THE UNIVERSITY *of* EDINBURGH

Methane cycling in upland soils of the Peruvian Andes and Amazon

Samuel Peter Jones

Doctor of Philosophy
University of Edinburgh
2014

Abstract

Significant discrepancies exist in global estimates of the atmospheric methane (CH_4) budget. This is particularly true for tropical South America where bottom-up approaches, rooted in field observation, tend to under estimate atmospheric observations. As such, a better understanding of soil environments, which are capable of acting as both source and sink for atmospheric CH_4, is required. Soil-atmosphere CH_4 exchange is fundamentally determined by the balance between strictly anaerobic methanogenic and aerobic methanotrophic microbial processes. For this reason, CH_4 emissions are typically associated with anoxic wetland soils, whilst, oxic upland soils are thought to uptake CH_4 from the atmosphere. However, there is increasing evidence that upland soils may act as sources of CH_4 through methanogenic activity within cryptic wetlands or anoxic microsites.

This thesis aims to: document soil-atmosphere CH_4 fluxes in poorly represented tropical upland and montane ecosystems, investigate controls on CH_4 flux with a focus on soil oxygen (O_2) concentration and investigate relationships between methanogenic and methanotrophic processes under oxic conditions. These aims are addressed in three chapters focusing on lowland terra firme, premontane and montane forests and montane humid puna grasslands and wetlands along an Amazonian to Andean transect spanning ~ 3300 m of elevation in southeastern Peru.

In the lowland rainforest intensive seasonal field campaigns and laboratory incubations were conducted on higher porosity ultisol and lower porosity inceptisol soils. Mean (s.e.) net CH_4 fluxes for dry and wet seasons were, respectively, -1.59 (0.06) and -1.39 (0.07) mg CH_4-C m^{-2} d^{-1} for the ultisol and -0.95 (0.06) and -0.41 (0.10) mg CH_4-C m^{-2} d^{-1} for the inceptisol. Greater uptake rates in the ultisol than the inceptisol were best explained by lower water-filled pore space (WFPS). Similarly, WFPS best explained between season variation in net CH_4 flux from the inceptisol, whilst, we were unable to explain the smaller variations observed for the ultisol. Methanogenic processes were active in both the ultisol and inceptisol soils despite oxic conditions.

In the premontane and montane forests, long-term monthly field measurements were conducted over two and a half years in premontane, lower montane and upper montane settings. Mean (s.e.) net CH_4 fluxes for aggregated dry and wet season months were, respectively, -0.20 (0.15) and -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} for the premontane forest, -1.12 (0.13) and -0.97 (0.11) mg CH_4-C m^{-2} d^{-1} for the lower montane forest and -1.55 (0.13) and -1.04 (0.11) mg CH_4-C m^{-2} d^{-1} for the upper montane forest. Increased uptake with elevation was best explained by decreases in WFPS. Significant variation in net CH_4 flux between seasons, driven by variation in WFPS, was only identified for the upper montane forest.

In the humid puna, upland ridge and slope and wetland depression and hollow features were studied through long-term monthly field measurements over two and a half years, intensive seasonal field campaigns and laboratory incubations. Mean (s.e.) net CH_4 fluxes for aggregated dry and wet season months were, respectively, -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} for the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} for the slope, -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} for the depression and 24.65 (10.70) and 181.74 (36.35) mg CH_4-C m^{-2} d^{-1} for the hollow. Variations in net CH_4 flux across the landscape were best explained by soil O_2 concentration. Methanogenic processes were active in these soils despite oxic conditions.

These data represent a key contribution in understanding soil CH_4 cycling in upland and montane soils of tropical South America. Humid puna landscapes, through the activity of both upland and wetland features, appear to have significant potential for CH_4 emission. In contrast, Andean humid tropical forests, similarly to their lowland counterparts, appear to principally uptake atmospheric CH_4. On balance, humid Andean regions may act as a source for atmospheric CH_4. Variations in soil O_2 concentration drive net CH_4 fluxes in upland humid puna grasslands through influence on the O_2 sensitivity of production. In contrast, uptake is insensitive to variations in soil O_2 concentration in settings where soil-atmosphere exchange is governed by high-affinity methanotrophy. In such situations variations are constrained by the influence of soil texture and water content on the inward diffusion of atmospheric CH_4.

Lay Abstract

Atmospheric methane (CH_4) is an important greenhouse gas. However, variations in the amount in the atmosphere over tropical regions like South America are poorly understood. Investigation of tropical soils, which can both produce and consume CH_4, may help explain these variations. Soils are thought to produce CH_4 when very wet conditions, in environments known as wetlands, reduce the movement of oxygen (O_2) from the atmosphere into the soil. This lack of O_2 is ideal for microbes, called methanogens, which breakdown organic matter and produce CH_4. Soils that are well drained, known as upland environments, are typically thought to uptake atmospheric CH_4 as microbes, called methanotrophs, consume O_2 and CH_4 moving into the soil. However, there is increasing evidence that well drained soils may also produce CH_4 owing to the presence of small sites of low O_2 availability.

This thesis aims to: measure CH_4 exchange between soil and atmosphere in poorly studied tropical upland and wetland ecosystems, investigate controls on this exchange and to investigate production and consumption of CH_4 in the presence of O_2. These aims are addressed in three chapters focussing on lowland terra firme rainforests, premontane and montane forests, and montane humid puna grasslands and wetlands, along an Amazonian to Andean transect spanning ~ 3300 m of elevation in southeastern Peru.

In the lowland forest, a less dense ultisol and more dense inceptisol soil were studied through intensive seasonal field campaigns and laboratory incubations. Mean (s.e.) net CH_4 exchange for the dry and wet season campaigns were, respectively, -1.59 (0.06) and -1.39 (0.07) mg CH_4-C m^{-2} d^{-1} for the ultisol and -0.95 (0.06) and -0.41 (0.10) mg CH_4-C m^{-2} d^{-1} for the inceptisol. Greater uptake rates in the ultisol than the inceptisol were best explained by lower water-filled pore space (WFPS). Similarly, WFPS best explained between season variation in exchange from the inceptisol, whilst, we were unable to explain the smaller variations observed for the ultisol. Production processes were active in both the ultisol and inceptisol soils despite oxic conditions.

In the premontane and montane forests, CH_4 exchange was studied in premontane, lower montane and upper montane settings through monthly field measurements over two and a half years. Mean (s.e.) net CH_4 exchange for dry and wet seasons were, respectively, -0.20 (0.15) and -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} for the premontane forest, -1.12 (0.13) and -0.97 (0.11) mg CH_4-C m^{-2} d^{-1} for the lower montane forest and -1.55 (0.13) and -1.04 (0.11) mg CH_4-C m^{-2} d^{-1} for the upper montane forest. Increased uptake rates with elevation were best explained by decreases in WFPS. Significant variation in CH_4 exchange between seasons, driven by variation in WFPS, was only identified for the upper montane forest.

In the humid puna, upland ridge and slope and wetland depression and hollow features were studied through monthly field measurements over two and a half years, intensive

seasonal field campaigns and laboratory incubations. Mean net methane exchange for dry and wet season were, respectively, -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} for the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} for the slope, -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} for the depression and 24.65 (10.70) and 181.74 (36.35) mg CH_4-C m^{-2} d^{-1} for the hollow. Variations in exchange across the landscape were best explained by soil O_2 concentration. Production processes were active in these soils despite oxic conditions.

These data represent a key contribution to the understanding of the role of tropical soils in controlling the amount of CH_4 in the atmosphere. Humid puna landscapes, through the activity of both upland and wetland features, appear to have significant potential for CH_4 emission. In contrast, Andean humid tropical forests, similarly to their lowland counterparts, appear to principally uptake atmospheric CH_4. On balance, montane landscapes in such humid Andean regions may act as a source of atmospheric CH_4. In the puna soil-atmosphere exchange is controlled by the sensitivity of methanogens to O_2 concentration, whilst, in the forests uptake is controlled by constraints on the inward movement of atmospheric CH_4 by soil texture and water content.

Declaration

I declare that this thesis was composed by myself and that the work contained therein is my own, except where explicitly stated otherwise in the text.

Samuel Peter Jones

Acknowledgements

Innumerable thanks are owed to:

Yit, Patrick, Dave and Niall for having the patience to let me learn from my mistake, being supportive when those mistakes didn't appear fixable and providing endless good advice,

Elaine and Anthony for help in the Drummond Street labs and Angus for tolerating my occupation of his lab in St Andrews,

Adan, Walter, William, Javier, Marco, Karol, Reymerth, Fernando, Jimmy, Beisit, Charol, Nelson, Cintia, Noah, Evan, Andy, Tor, Vika and Graham for assistance and friendship during long periods of field and lab work,

Bron, JC, Gary, Conan, Tim, Luke, Abbie, Iain, Tom, Will, Matt and Clode for things too numerous to list,

St Andrews Tourist Hostel and the sadly deceased Southern Comfort Hostel for shelter and fleeting times with many excellent people - hopefully we'll cross paths again soon.

Tread Lightly, Speak Dearly

On Lightyear's Call of the Weasel Clan, 2001

Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 The global atmospheric CH_4 budget

Methane (CH_4) is a potent greenhouse gas and reactive component of the atmosphere (Cicerone and Oremland, 1988). The concentration of atmospheric CH_4 (Figure 1.1), driven by changes in anthropogenic activity, increased from 715 ± 4 to 1774 ± 1.8 parts per billion by volume (ppb) between 1750 and 2005 (Forster et al., 2007). Increased absorption of long-wave radiation associated with this growth directly contributed 0.48 ± 0.05 to a total radiative forcing for the period of 2.63 ± 0.26 $\mathrm{W\,m^{-2}}$. Additional indirect radiative effects resulting from oxidation of CH_4 by hydroxyl radicals, through feedback between hydroxyl radical concentration and atmospheric residence time of CH_4, production of carbon dioxide (CO_2) in the terminal step of oxidation and formation of both tropospheric ozone and stratospheric water vapour, contributed a further 0.22 to 0.38 $\mathrm{W\,m^{-2}}$ (Hansen et al., 2000; Shindell et al., 2005). As such, CH_4 is the second most important anthropogenic greenhouse gas after CO_2.

Globally the contemporary atmospheric CH_4 budget consists of sources to the atmosphere of 503 to 610 Tg CH_4 yr^{-1} and sinks from the atmosphere of 428 to 507 Tg CH_4 yr^{-1} (Denman et al., 2007). Thus, a dynamic imbalance of 11 to 33 Tg CH_4 yr^{-1} exists in contemporary budgetary estimates. For this reason, the growth rate of atmospheric CH_4 concentration is characterised by significant inter and intra annual variability (Figure 1.2). Decadal trends indicate a decrease from an average growth

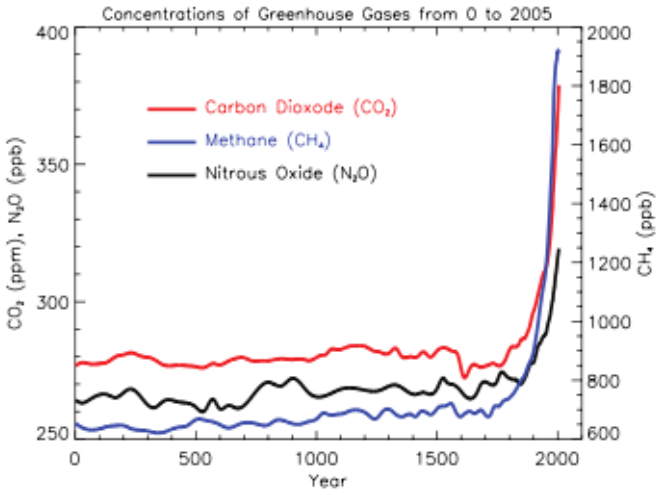

Figure 1.1: The atmospheric concentration of the greenhouse gases CO_2 (ppm), CH_4 (ppb) and nitrous oxide (ppb) between 0 and 2005 AD. The concentration of atmospheric CH_4 increased from a pre-industrial value of ~ 715 to 1774 ppb in 2005. This increase is largely attributed to anthropogenic changes in agriculture, fossil fuel exploitation and waste management (image: Forster et al. (2007))

.

rate for atmospheric CH_4 of ~ 12 ± 2 ppb yr^{-1} in the 1980s to a relatively stable ~ 4 ± 4 ppb yr^{-1} during the early 21^{st} century (Dlugokencky et al., 2003; Bousquet et al., 2006). Notably, since 2007 the growth rate has once again increased (Kirschke et al., 2013). These variations in growth rate reflect a composite of trends in the strength of processes acting as sources and sinks. Inter annual differences in growth rate are attributed to variability in the strength of natural components (Dlugokencky et al., 2009). Similarly, the long term trend in growth rate is attributed to decreased anthropogenic emissions during the late 20^{th} century (Dlugokencky et al., 2003; Bousquet et al., 2006; Kirschke et al., 2013). The relative contributions of anthropogenic and natural emissions driving variations observed in the 21^{st} century are less clear . In this respect, understanding the drivers of variability in the strength of CH_4 sources and sinks is required in attributing contemporary growth rate trends and assessing the implications of global climate change for future atmospheric CH_4 concentrations (Nisbet et al., 2014). In both of these contexts, constraining the coupling between climatic variations and strength of natural sources and sinks is of particular importance as feedbacks between these components represent a central uncertainty in modelling CH_4 cycle dynamics (Nisbet et al., 2014). Indeed, interest in such feedbacks has been renewed, fuelled by uncertainty in the sensitivity of biological processes to changes in temperature and precipitation (Bardgett et al., 2008; O'Connor et al., 2010; Singh et al., 2010), speculation

concerning Arctic permafrost thaw and CH_4 hydrate dissociation (Walter et al., 2006; Nisbet and Chappellaz, 2009; Shakhova et al., 2010), evidence that the tropics act as a larger source than previously thought (Mikaloff Fletcher et al., 2004a,b; Frankenberg et al., 2005; Bloom et al., 2010b) and identification of potentially important and unaccounted for soil, plant, and aquatic sources of atmospheric CH_4 (Spahni et al., 2011; Keppler et al., 2006; Martinson et al., 2010).

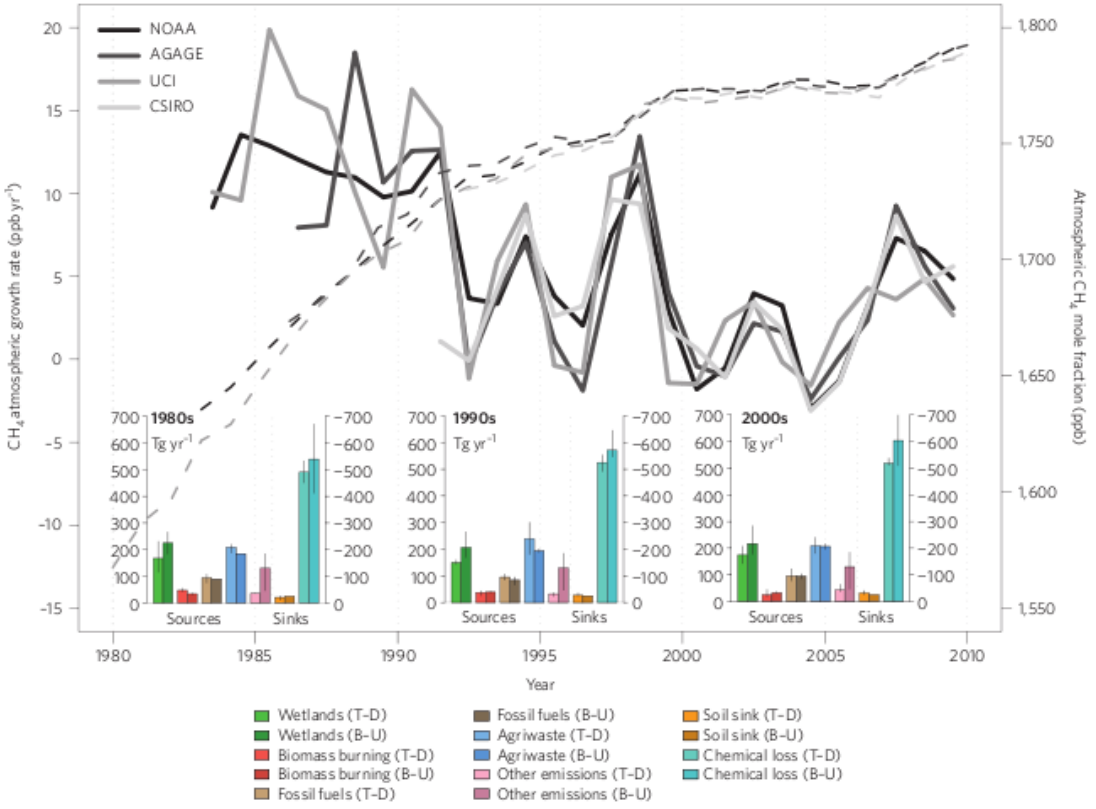

Figure 1.2: Global atmospheric CH_4 concentration (dashed lines) and growth rates (solid lines) from various sampling networks and bottom-up (B-U) and top-down (T-D) estimates of the magnitude of various sources and sinks of atmospheric CH_4 for the 1980s, 1990s and 2000s atmospheric (image: Kirschke et al. (2013))

.

1.2 Soils as sources and sinks for atmospheric CH_4

The relative contribution of different sources and sinks to the global atmospheric CH_4 budget is constrained by scaling inventories of sources and sinks through top-down inverse modelling simulations utilising regional measurement of the concentration and isotopic composition of atmospheric CH_4 and bottom-up estimates of source and sink

strength based on direct measurements and process models (O'Connor et al., 2010). Sources of atmospheric CH_4 are diverse (Denman et al., 2007). Anthropogenic activity such as rice agriculture, fossil fuel exploitation, ruminant husbandry, disposal of organic waste and biomass burning, emits 264 to 428 Tg CH_4 yr^{-1} to the atmosphere (Denman et al., 2007). Natural sources such as soils, termites, oceans, hydrate dissociation, geological seeps, wild ruminants and wildfires emit a further 145 to 222 Tg CH_4 yr^{-1} (Denman et al., 2007). Of these, natural soil environments, accounting for ~ 20 to 40 $\%$ of natural sources, are the single most significant contributor (Denman et al., 2007). Soil sources are typically associated with wetland environments where water tables close to the surface promote conditions required for methanogenic microbial communities involved in CH_4 production (Conrad, 1996). Sinks of atmospheric CH_4 are dominated by the oxidisation of 428 to 507 Tg CH_4 yr^{-1} by hydroxyl radicals in the lower troposphere (Denman et al., 2007). Escape to the stratosphere accounts for a further 30 to 45 Tg CH_4 yr^{-1} and 26 to 43 Tg CH_4 yr^{-1} is taken up in soils (Denman et al., 2007). Soil environments represent only ~ 5 - 6 $\%$ of the total atmospheric CH_4 sink; however, they are the only significant known biological sink (Denman et al., 2007). Soil sinks are typically associated with well aerated environments, broadly refered to as upland soils, where the absence of significant saturation promotes conditions required for methanotrophic microbial communities involved in CH_4 oxidation (Conrad, 1996). Methanotrophic activity also consumes the majority of CH_4 produced within wetland soils in situ, thus, precluding a larger soil source (Segers, 1998). In this respect, natural soil environments are quantitatively important in the global CH_4 cycle as both source and sink of atmospheric CH_4.

The importance of the coupling between climate and soils in determining the concentration of atmospheric CH_4 is well established. During the late Pleistocene, the atmospheric concentration of CH_4, as determined from ice core records spanning 800 kyr, varied between ~ 300 and 800 ppb (Spahni et al., 2005; Loulergue et al., 2008). These variations appear to largely reflect the response of wetlands to the ~100 kyr periodicity in the Earth's orbit, with concentration minima and maxima occurring during glacial and inter-glacial periods, respectively (Loulergue et al., 2008; Fischer et al., 2008). An increased contribution of orbital precession, with a periodicity of 23 kyr, to this sig-

nal over the last four glacial cycles is attributed to greater insolation at northern low latitudes leading to increased tropical precipitation and subsequent wetland expansion (Loulergue et al., 2008; Wolff, 2011). Singarayer et al. (2011) are able to successfully reproduce these trends for the last 130 kyr using a general circulation model to drive coupled global vegetation - wetland emission models. Notably, this approach is able to replicate and attribute poorly understood mid-Holocene increases in atmospheric CH_4 concentration to modification of precipitation patterns and increased emissions, particularly from South America, in the Southern Hemisphere tropics.

Similarly, inter annual variations in the growth rate of atmospheric CH_4 concentration during the late 20^{th} and early 21^{st} centuries appear to be particularly sensitive to the response of wetlands to climate. This period (Figure 1.2) is characterised by short term positive growth rate excursions, on the order of ~ 10 ppb, occurring with a periodicity of 7.7 yr and considerable inter annual variability around the decadal trend (Khalil et al., 2007). Bousquet et al. (2006) suggest that 70 $\%$ of the growth rate signal, between 1984 and 2003, is accounted for by variability, particularly in the tropics and Southern Hemisphere, in wetland source strength. This variation is broadly attributed to the extent of seasonally inundated, tropical wetlands as driven by precipitation and the strength of emissions in perennial, higher latitude wetlands as driven by temperature (Walter and Heimann, 2000). Indeed, this relationship appears to explain considerable growth rate excursions observed in 1997 - 1998 and 2007 - 2008. The increase in growth rate to 6.3 ± 0.7 ppb in 1997 is attributed to greater biomass burning during a strong El Niño-Southern Oscillation episode (Bousquet et al., 2006; Dlugokencky et al., 2009). The subsequent increase in growth rate to 12.4 ± 0.7 ppb in 1998 is attributed to the expansion of tropical wetlands as a result of intensified precipitation associated with the transition into a La Niña episode (Mikaloff Fletcher et al., 2004a; Bousquet et al., 2006; Chen and Prinn, 2006; Dlugokencky et al., 2009). Similarly, growth-rates of 8.3 ± 0.6 ppb in 2007 and 4.4 ±- 0.6 ppb in 2008 are, respectively, associated with El Niño conditions and a La Niña episode (Rigby et al., 2008; Dlugokencky et al., 2009). Greatest increases in emissions are observed over northern polar latitudes and the Southern Hemisphere in 2007 and the tropics in 2008; associated, in the first instance, with elevated Arctic temperatures and possible decreases in the at-

mospheric hydroxyl sink, and, in the second instance, with above average precipitation in the Amazon and Southeast Asia.

1.3 Discrepancy within the South American atmospheric CH_4 budget

At temperate and polar latitudes, bottom-up estimates tend to overestimate the magnitude of wetland sources when compared to upper constraints implied by top-down approaches (Mikaloff Fletcher et al., 2004a; Bloom et al., 2010b; Kirschke et al., 2013). However, for tropical regions like South America top-down estimates tend to indicate a greater source of atmospheric CH_4 than implied by bottom-up approaches (Mikaloff Fletcher et al., 2004b; Frankenberg et al., 2008; Bloom et al., 2010b). For example, Bloom et al. (2010b) find the majority of variability in atmospheric CH_4 concentration, between 2003 and 2005, to be explained by water table depth in the tropics and temperature at higher latitudes, as inferred from satellite observations of solar-back scatter radiation, gravity anomalies and land-surface temperature. Whilst a remarkably strong relationship, for a study with a spatial resolution of 3° of latitude and longitude, is identified between water table depth and atmospheric CH_4 concentration in the tropics, a notable discrepancy exists for the Amazon basin. In contrast to other major tropical river basins, such as the Niger, Ganges and Congo, no significant relationship is found for the Amazon basin, with peak atmospheric CH_4 concentration preceding peak water-table depth by 1 to 3 months. Considering that the tropics of this region account for ~ 10 to 15 $\%$ of the global atmospheric CH_4 source budget and seem to play a central role in driving Quaternary variations atmospheric growth rate, understanding the distribution and controls on source-sink activity for this region is of global significance (Mikaloff Fletcher et al., 2004a,b; Frankenberg et al., 2008; Bloom et al., 2010b).

Source-sink inventories in tropical South America have focussed on the Amazon basin with uptake associated with upland soils of extensive lowland terra firme rainforests (Potter et al., 1996; Dutaur and Verchot, 2007) and emissions associated with a variety

of wetlands (Melack et al., 2004; Ringeval et al., 2010; Bloom et al., 2012). Discrepancies in both the magnitude of budgetary estimates and the expected drivers of source strength suggests that the seasonal development of expansive, inundated soils in the Amazon basin does not adequately characterise the South American CH_4 cycle. In this context, it seems likely that less well constrained sources such as undocumented wetland soils in upland settings (McClain et al., 2003; Josse et al., 2009a) or anaerobic microsites within upland soils (Silver et al., 1999), aerobic UV-irradiation of foliar pectin (McLeod et al., 2008) and novel aquatic environments such as tank bromeliads (Martinson et al., 2010), may make key contributions. Indeed, such processes may account for unattributed CH_4 sources observed in terra firme tropical forest environments (do Carmo et al., 2006; Miller et al., 2007; Sinha et al., 2007). The contribution of such processes, hampered by limited understanding of their environmental controls, to the atmospheric CH_4 budget is poorly constrained. For example, Bloom et al. (2010a) and Martinson et al. (2010) estimate that pectin degradation and tank bromeliads globally act as relatively small sources of CH_4 to the atmosphere of 0.2 to 1.0 Tg CH_4 yr^{-1} and 1.2 Tg CH_4 yr^{-1}, respectively. Contrastingly, Spahni et al. (2011) indicate that wet upland soils could represent a source, globally emitting 60 to 90 Tg CH_4 yr^{-1}, of considerable significance. Such activity in upland soils, which in South America are typically considered to account for 10 to 20 $\%$ of the global soil sink, may help to explain the observed regional discrepancies (Potter et al., 1996; Dutaur and Verchot, 2007; Spahni et al., 2011). Similarly, transient or localised inundation in upland ecosystems may represent hotspots of CH_4 emission not currently considered in budgetary estimates (Waddington and Roulet, 1996; McClain et al., 2003).

Soil emissions of CH_4 have been observed in a variety of temperate and tropical upland ecosystems (Spahni et al., 2011). Emissions of CH_4 from upland soils may be particularly prevalent in highland regions of humid tropics where the combination of organic rich soils, abundant precipitation and relatively warm temperatures are suited to such activity (Silver et al., 1999; Schuur et al., 2001; Von Fischer and Hedin, 2002; Teh et al., 2005). For example, tropical montane forest soils in Puerto Rico emit CH_4 at similar rates to that observed in wetland environments and contrast weak source and uptake activity in proximal premontane and lowland forest soils (Silver et al., 1999;

Teh et al., 2005). Despite this, there are relatively few studies of upland soil CH_4 cycling in lowland Amazonia (Keller et al., 1986; Steudler et al., 1996; Verchot et al., 2000; Palm et al., 2002; Fernandes et al., 2002; Davidson et al., 2004; Keller et al., 2005; Davidson et al., 2008; Neto et al., 2011) and a distinct paucity in the highlands (Neto et al., 2011; Wolf et al., 2012). Similarly, the presence of montane wetlands in the tropical Andes is well documented but soil-atmosphere CH_4 exchange from these ecosystems has not been characterised (Wania et al., 2009; Josse et al., 2009a). In these respects, in addition to further characterisation of the activity of swamp forests, seasonally inundated floodplain forests, rivers and lakes in lowland Amazonian (Devol et al., 1988; Richey et al., 1988; Devol et al., 1990; Bartlett et al., 1990; Engle and Melack, 2000; Smith et al., 2000b; Lima, 2005; Marani and Alvala, 2007; Bastviken et al., 2010; Belger et al., 2011; Sawakuchi et al., 2014), consideration of the source potential of upland soils in both the lowlands and highlands of tropical South America is required.

1.4 Soil methanogenesis and methanotrophy

Soils act as both a source and sink for atmospheric CH_4 through its production and consumption by microbial communities (Conrad, 1996). In the first instance, CH_4 is produced by obligate anaerobic methanogenic archaea; a process known as methanogenesis. In the second instance, CH_4 produced through in situ methanogenesis or that diffusing inwards from the atmosphere is consumed by aerobic methanotrophic bacteria; a process known as methanotrophy.

In the majority of soil environments methanogenic metabolisms tend to operate through aceticlastic or hydrogentrophic pathways (Conrad, 1999). Hydrogentrophic methanogens (Equation 1.1), belonging to the orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanopyrales*, reduce CO_2 or bicarbonate to CH_4 through the coupled oxidation of hydrogen as an electron donor (Ferry, 1999; Liu and Whitman, 2008). Aceticlastic methanogens (Equation 1.2) cleave acetate through fermentation, reducing methyl groups to CH_4 and oxidising carboxyl groups

to CO_2 (Ferry, 1993).This activity is a form of methylotrophy, generally associated with the orders *Methanosarcinales* and *Methanobacteriales*, utilised by the genera *Methanosarcina* and *Methanosaeta* of the *Methanosarcinales* (Liu and Whitman, 2008). Broadly methylotrophs are capable of reducing the methyl groups of methylated compounds such as methanol, methylated amines and methylated sulphides to CH_4 using electrons liberated from the oxidisation of additional methyl groups to CO_2 (Hornibrook and Aravena, 2009). However, such metabolic activity is relatively uncommon in soils compared to hydrogentrophic and aceticlastic pathways which are principally constrained by the availability of hydrogen and acetate (Conrad, 1999). The supply of these substrates involves a number of syntrophic microbial groups which act to break down complex organic molecules; namely, hydrolytic, acidogenic and acetogenic bacteria (Le Mer and Roger, 2001; Drake et al., 2009; Hornibrook and Aravena, 2009). Hydrolytic bacteria, utilising aerobic, facultative or obligate anaerobic metabolisms, hydrolyse large organic polymers into monomers such as glucides, fatty acids and amino acids (Le Mer and Roger, 2001). Acidogenesis driven by facultative or obligate anaerobic bacteria utilises these monomers and other intermediates to produce long-chain volatile fatty acids, alcohols, hydrogen, CO_2, formate and acetate (Le Mer and Roger, 2001; Drake et al., 2009; Hornibrook and Aravena, 2009). Higher long-chain volatile fatty acids and alcohols are further oxidised to acetate and hydrogen or formate by homoacetogenic or syntrophic acetogenic bacteria (Hornibrook and Aravena, 2009). Notably, both aerobic and anaerobic microbial communities utilising more thermodynamically favourable electron acceptors, such as oxygen (O_2), nitrate, ferric iron, manganese (IV), and sulphate, also utilise methanogenic precursors, acetate and hydrogen for metabolic purposes (Lovley et al., 1982; Lovley and Phillips, 1987; Hall et al., 2013). In this respect, methanogenesis represents the terminal step in soil carbon mineralisation once more rewarding pathways are exhausted.

$$CO_2 + 4H_2 \rightarrow 2H_2O + CH_4 \tag{1.1}$$

$$CH_3COOH \rightarrow CO_2 + CH_4 \tag{1.2}$$

Aerobic methanotrophs (Equation 1.3) are a group of methylotrophic gram-negative bacteria that catalyse the oxidisation of CH_4 to CO_2 through the reduction of O_2 (Conrad, 1996). This activity accounts for 2 to 7 $\%$ of the global atmospheric CH_4 sink and consumes 10 to 90 $\%$ of endogenous CH_4 in situ (Denman et al., 2007; Segers, 1998). The exploitation of these different sources highlights a significant functional distinction within the group; namely, the activity of high-affinity methanotrophs capable of metabolising CH_4 at atmospheric concentrations and low-affinity methanotrophs requiring elevated concentrations of CH_4 (Bender and Conrad, 1992; Conrad, 1996). The transition between the activity of these groups is variable but broadly occurs over CH_4 concentrations on the order of 100 to 1000 ppm (Bender and Conrad, 1992, 1995; Gulledge and Schimel, 1998; Reay and Nedwell, 2004). Whilst, low affinity methanotrophs consist of a broad group belonging to the orders *Gammaproteobacteria*, *Alphaproteobacteria* and *Verrucomicrobi*, the methanotrophic communities involved in activity at atmospheric CH_4 concentrations have proved more difficult to isolate (Knief et al., 2003; Kolb et al., 2005; Op den Camp et al., 2009; Kolb, 2009). Isolated methanotrophs typically catalyse the oxidation of CH_4 using particulate and soluble monooxyenase enzymes and assimilate carbon via formaldehyde ribulose monophosphate or serine cycle pathways (Conrad, 1996; Hanson and Hanson, 1996; Chistoserdova et al., 2005; Dedysh et al., 2000; Chistoserdova et al., 2009; Op den Camp et al., 2009). Evidence that some of these methanotrophs are able to oxidise CH_4 at low concentrations through a secondary enzyme system and that some are capable of heterotrophic activity may hint at an explanation for the activity of high affinity communities (Dedysh et al., 2005; Baani and Liesack, 2008; Conrad, 2009).

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \tag{1.3}$$

1.5 Soil O_2 as a driver of soil-atmosphere CH_4 exchange

The function of a soil environment as source or sink is determined by the net balance between gross rates of methanogenic and methanotrophic processes (Figure 1.3). The disparate O_2 requirements of the microbial communities driving these processes results in the general assumption that, through slower liquid relative to gas phase rates of diffusion, inundated soil environments, which promote anoxic conditions, act principally as net sources, whereas, non-flooded soil environments, which promote oxic conditions, act principally as net sinks (Conrad, 1996). Subsequently, in this thesis soil environments typified by innudation such as swamps and bogs are refered to as wetlands, whilst, freely draining environments are refered to as uplands (Conrad, 1996). In this context, the term upland is used as a generalised indicator of topographic position within an environment whilst the terms lowland, highland and montane are used to refer to position with respect to elevation. For example within lowland Amazonia, the soils of terra firme rainforest growing on raised terraces are refered to as upland soils in order to distinguish these environments from the soils of wetland swamp forests or those of seasonally innudated flood plain forests within proximal topographic lows (Verchot et al., 2000). As such, emission and uptake of CH_4 by soils is usually attributed to wetland and upland soils, respectively. However, methanotrophic activity can be significant in wetland soils through occupation of surficial oxic layers or the rhizospheres of aerenchymatous plants and similarly anaerobic processes such as methanogenesis operate in oxic soils through inhabitation of localised microsites of anoxia. Understanding the controls on methanotrophic and methanogenic processes under such differing conditions is central in constraining soil-atmosphere CH_4 exchange.

Well aerated upland soils act to uptake atmospheric CH_4 through the activity of high-affinity methanotrophic communities (Bender and Conrad, 1992). In such soils methanogenesis and low-affinity methanotrophy are not expected to play important roles in the soil CH_4 cycle as soil O_2 concentrations close to atmospheric levels preclude significant production of CH_4 (Conrad, 1996; Kursar et al., 1995; Silver et al., 1999; Cleve-

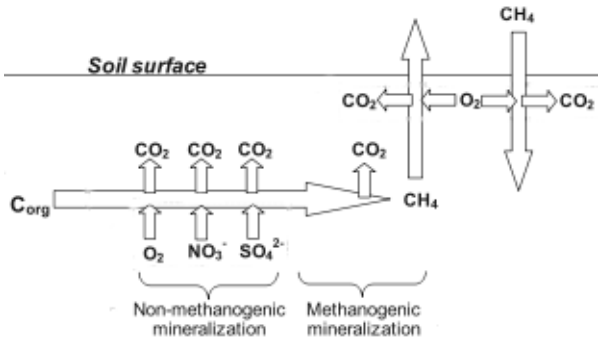

Figure 1.3: Fundamentally soil CH_4 cycling is the result of methanogenic production, as constrained by the availability of substrates, and the methanotrophic consumption constrained by the availability of CH_4 and O_2. In soils that act as sinks for atmospheric CH_4, variations in uptake result from constraints on the inward diffusion of CH_4 from the atmosphere. Contrastingly in soils where significant methanogenesis occurs, soil-atmosphere exchange is a function of the balance between the rate of production and consumption of endogenous CH_4. If the capacity for low-affinity methanotrophy to consume endogenous CH_4 is greater than its rate of production, soils may still act as a sink for atmospheric CH_4 through the activity of high-affinity methanotrophs. Alternatively, rates of production may exceed the rate of consumption and soils will act as a source for atmospheric CH_4. In such situations soil-atmosphere CH_4 exchange will vary in response to constraints on the rate of methanogenic mineralisation of substrates (image: Von Fischer and Hedin (2007))

.

land et al., 2010). For example, Silver et al. (1999) report a mean uptake rate of -0.36 (0.05) mg CH_4-C m^{-2} d^{-1} for a low elevation upland tropical forest soil with an O_2 concentration of 21 (0.03) $\%$ in the upper 35 cm. Under such conditions, O_2 is abundant and methanotrophic oxidation is expected to be limited by the availability of CH_4 (Bender and Conrad, 1992). In this respect, variations in soil-atmosphere CH_4 arise from variations in soil texture and water content, typically reflected in measures of water filled pore space, through constraints on the diffusional ingress of atmospheric CH_4 (Smith et al., 2000a, 2003; Dutaur and Verchot, 2007) (Figure 1.4). Due to their reliance on the diffusional supply of atmospheric CH_4 communities of high-affinity methanotrophs occupy well connected pore spaces in surficial soils (Von Fischer et al., 2009).

In contrast, wetland soils are broadly expected to account for soil sources of methane to the atmosphere as a result of methanogenic activity in anoxic zones formed under saturated conditions (Conrad, 1996). In this respect, vertical stratification of oxic and anoxic conditions occurs across the water table as biological oxygen demand is greater than the rate of diffusion across the liquid-gas interface (Figure 1.4). Subsequently energetically favourable electron acceptors are depleted and methanogenesis occurs at

depth. Low-affinity methanotrophy plays an active role in the CH_4 cycle of wetland soils utilising the abundance of CH_4 and O_2 close the surface of the water table or in oxygenated rhizospheres (Segers, 1998). In this context, variations in net CH_4 fluxes from wetland soils are typically related to water table depth as a measure of anoxic and oxic conditions controlling the balance between methanogenic and methanotrophic activity (Turetsky et al., 2014).

However, microbes with facultative and obligate anaerobic metabolisms are also found in upland soils (Peters and Conrad, 1996; Megonigal and Guenther, 2008). Furthermore, both in vitro and in situ observations indicate that obligate anaerobic and aerobic processes can operate simultaneously in such settings irrespective of the presence of O_2 in the bulk soil atmosphere (Silver et al., 1999; Von Fischer and Hedin, 2002; Teh et al., 2005; Liptzin and Silver, 2009; Liptzin et al., 2011) (Figure 1.4). In this context, methanogenesis occurs within anoxic microsites formed in response to physical limitations on the diffusion of O_2 into soil pores occluded by saturation or within aggregates and the in situ depletion of O_2 through aerobic respiration (Sexstone et al., 1985; Verchot et al., 2000; Teh and Silver, 2006). Similarly to wetland settings, low-affinity methanotrophy, utilising abundant O_2 and elevated CH_4 concentrations proximal to zones of production, is associated with this activity (Von Fischer and Hedin, 2002; Teh et al., 2005). Whilst sporadic emissions in both space and time are commonly observed from many upland soils that principally act as sinks for atmospheric CH_4, these processes may be particularly pertinent in humid tropical settings where the combination of high precipitation and warm temperatures support wet, biologically active soils suited to driving soil O_2 concentrations to sub-atmospheric levels (Silver et al., 1999; Smith et al., 2000a; Cleveland et al., 2010). For example, Silver et al. (1999) report mean emissions of 0.24 to 73.25 mg CH_4-C m^{-2} d^{-1} from tropical forest soils with O_2 concentrations between 13 and 6 $\%$.

In these respects, measurements of soil O_2 concentration may represent a useful tool for investigating variations in soil-atmosphere CH_4 exchange in upland soils where soil CH_4 cycling may not be limited to high-affinity methanotrophic activity. Despite this, soil O_2 concentration is rarely reported in studies of tropical soils (Kursar et al., 1995;

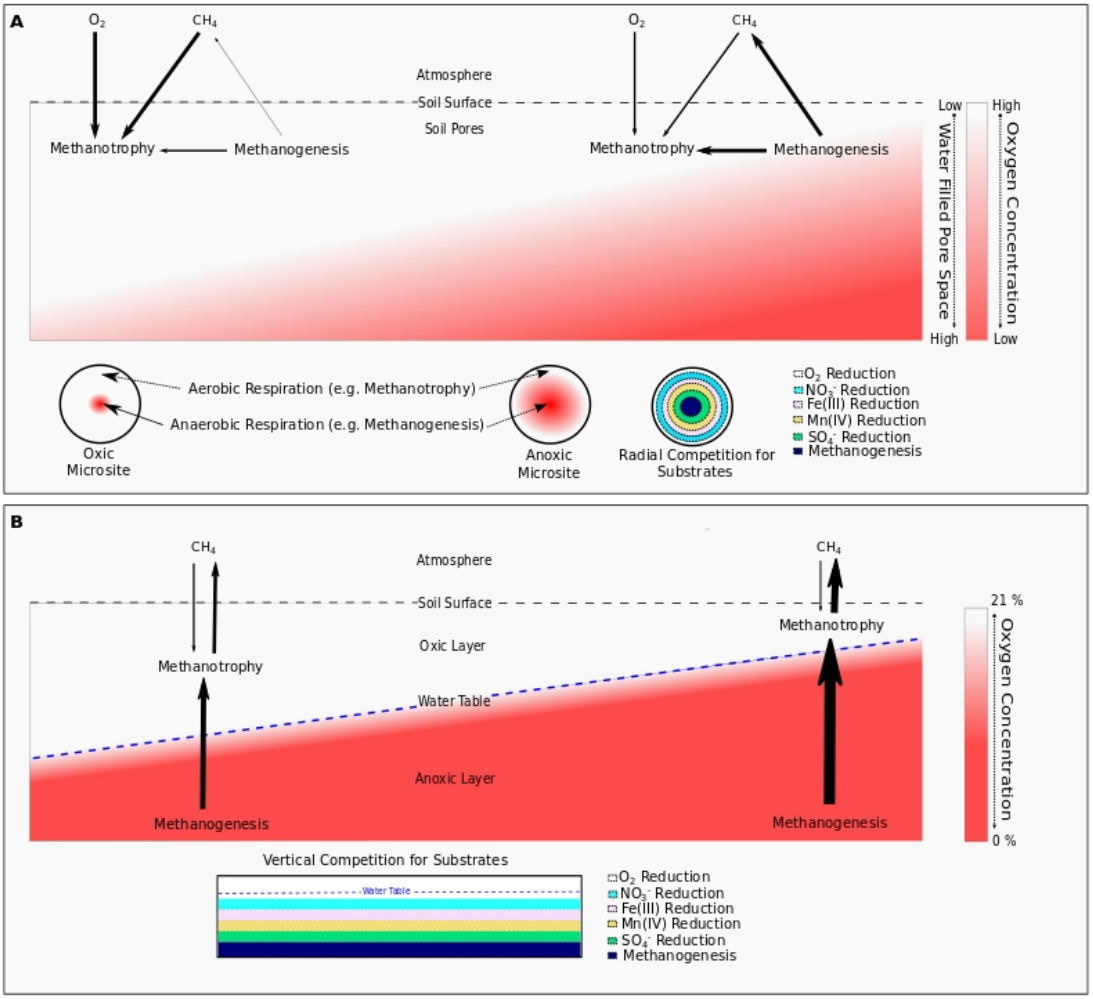

Figure 1.4: Conceptual depictions of net CH_4 exchange in upland (A) and wetland (B) soil settings. A: In upland settings the diffusion of atmospheric CH_4 and O_2 into the soil supports the activity of high-affinity methanotrophic communities, inhabiting well-connected oxic pore spaces, that facilitate the uptake of atmospheric CH_4. Anaerobic microbial metabolisms may also be active in such settings but are likely to be relatively limited when soils are well aerated. As water filled pore space increases the inward diffusion of gases from the atmosphere is restricted constraining the supply of atmospheric CH_4 to methanotrophic communities and promoting anaerobic microbial activity as anoxic microsites develop. In this way positive relationships between net CH_4 flux and water filled pore space may result from some combination of the relative inhabitation and promotion of methanotrophic and methanogenic microbial communities, respectively. In the latter case, changes in methanogenic activity in response to variations in the developement of anoxia within microsites is dependant on the presence of more energetically favourable electron acceptors. B: In wetland soils oxic and anoxic zones are principally delineated by the presence of the water table with CH_4 produced below the water table consumed by methanotrophy in overlying oxic layers. As the water table approaches the soil surface methanogenesis is promoted, depending on the presence of alternative electron acceptors, relative to methanotrophy and net CH_4 fluxes increase.

Silver et al., 1999; Schuur et al., 2001; Cleveland et al., 2010). Indeed, the majority of work focusing on the relation between soil CH_4 cycling and the distribution of soil O_2 has focussed on tropical forests of Puerto Rico (Silver et al., 1999; Teh et al., 2005; Liptzin et al., 2011; Hall et al., 2013).

1.6 Relationships between soil methanogenesis and methanotrophy

In soils that principally act to uptake atmospheric CH_4, soil-atmosphere exchange is expected to respond to variations in the activity of high-affinity methanotrophic communities, principally through the influence of diffusional CH_4 supply or water stresses (Del Grosso et al., 2000; Smith et al., 2003; Von Fischer et al., 2009). However, in soils with significant anaerobic activity variations in soil-atmosphere CH_4 exchange, modulated by differences in transport associated with diffusion, vegetation and ebullition, result from the balance between methanogenic and methanotrophic processes (Le Mer and Roger, 2001). In this context, the sensitivity of soil-atmosphere exchange to the individual activity of gross processes of production and consumption has implications for constraining the response to variations in the extent of anoxic and oxic conditions (Hall et al., 2013). That is, soil-atmosphere CH_4 exchange may be predominantly controlled by variations in production or consumption (Von Fischer and Hedin, 2007).

Consumption may control soil-atmosphere exchange in situations where methanotrophic potential is such that it exceeds variations in methanogenic production (Del Grosso et al., 2000; Von Fischer and Hedin, 2007). For example, in settings where zones of production and oxidation are vertically stratified, highly active oxic layers at the surface may preclude emissions (Fritz et al., 2011). Methanotrophy is micro-phillic with evidence to suggest that rates of oxidation are insensitive to variations in O_2 concentration above 3 $\%$ (Bender and Conrad, 1994; Teh et al., 2006). In this respect, whilst variations in water table depth in wetland settings may be sufficient to suppress methanotrophic activity, this is less likely in upland soils where despite anoxic conditions within microsites the bulk soil environments are typically oxic (Silver et al., 1999;

Schuur et al., 2001; Cleveland et al., 2010). In this context, whilst consumption may track the rate of production, source activity is ultimately determined by the response of production to variations in the extent of anoxic conditions.

Production may control variations in soil-atmosphere exchange in situations where variations in anoxia allow methanogenic activity to outstrip oxidation (Von Fischer and Hedin, 2007). For example, Teh et al. (2005) apply an isotopic mass balance model to differences in net CH_4 flux between two years in a Puerto Rican montane forest and suggest an increase in gross CH_4 production of 94 $\%$ is accompanied by a lesser increase in consumption of 20 $\%$. In this respect, the greater temperature sensitivity of methanogenesis than methanotrophy and constraints on the supply of substrates to methanogenic communities may represent significant controls on the response of such soils to environmental conditions (Segers, 1998; Von Fischer and Hedin, 2007; Teh et al., 2008a; Dubinsky et al., 2010). For example, Von Fischer and Hedin (2007) use an isotope pool dilution technique to partition gross CH_4 production and consumption for surface soils along a Hawaiian montane rainfall gradient and find that net CH_4 emission, occurring between water filled pore spaces of 60 to 90 $\%$, is only observed if the methanogenic fraction of total soil carbon mineralization exceeds 0.04 $\%$. Such constraints on CH_4 production are supported by observation of thresholds between uptake and emission of CH_4 in upland soils where iron reduction competitively suppresses methanogenesis (Dubinsky et al., 2010).

1.7 Aims and approach

Better characterisation of the magnitude and controls on soil-atmosphere CH_4 exchange from South American humid tropical upland ecosystems may help to improve agreement between top-down and bottom-up estimates of the regions atmospheric CH_4 budget. In this context, we address these issues in terms of the dominant biomes found along the Andes Biodiversity and Ecosystems Research Group (ABERG) elevation transect in southeastern Peru (Malhi et al., 2010). The transect spans the transition from lowland Amazonian to the high Andes over ~ 3300 m of elevation. In this region,

biomes broadly transition from lowland rainforest and savannah (< 600 m above sea level (asl)), through premontane rainforest (800 - 1200 m asl), lower (1200 - 2200 m asl) and upper montane cloud forests (2200 - 3400 m asl) to montane grasslands known as puna (> 3400 m asl) (Girardin et al., 2010). The ABERG elevation is broadly representive of the dominant environments of tropical South America with lowland humid tropical rainforest covering ~ 30 % of the continent (Eva et al., 2004; Malhi et al., 2010). Similarly, humid tropical montane forests and grasslands respectively cover ~ 19 and 23 % of the tropical Andes which as a region accounts for ~ 7 % of continental land-cover (Eva et al., 2004; Tovar et al., 2013). For this reason, the transect has previously been the focus of in depth studies of both plant and soil carbon cycling, however, soil CH_4 cycling has not previously been addressed (Girardin et al., 2010; Zimmermann et al., 2009, 2010b,a; Gibbon et al., 2010; van de Weg et al., 2012; Fisher et al., 2013; Whitaker et al., 2014; Oliveras et al., 2014a).

To address the gap in knowledge for the transect and the more general requirement for better characterisation of upland soil CH_4 cycling in both lowland and montane settings in tropical South America, this thesis focuses on lowland terra firme rainforest at 200 m asl (Tambopata: $12°49'50''$ S, $69°16'11''$ W), premontane rainforest at 1000 m asl (Hacienda Villa Carmen: $12°49'50''$ S, $69°16'11''$ W), lower montane cloud forest at 1500 m asl (San Pedro: $13°02'56''$ S, $71°32'13''$ W), upper montane cloud forest at 3030 m (Wayqecha: $13°11'24''$ S, $71°35'13''$ W) and montane humid puna grassland at 3500 m asl (Tres Cruces: $13°07'19''$ S, $71°36'54''$ W). The premontane, lower and upper montane forest and puna grasland sites are located in or above the Kosnipata valley, Paucartambo, Department of Cusco, whilst, the lowland rainforest site is located in the Tambopata National Reserve, Tambopata Province, Department of Madre de Dios, Peru (Figure 1.5).

This thesis aims to:

1. To characterise soil-atmosphere CH_4 exchange for upland soils in a variety of poorly characterised tropical South American ecosystems. Upland soils are typically expected to act as a sink for atmospheric CH_4, however, the prevalence of

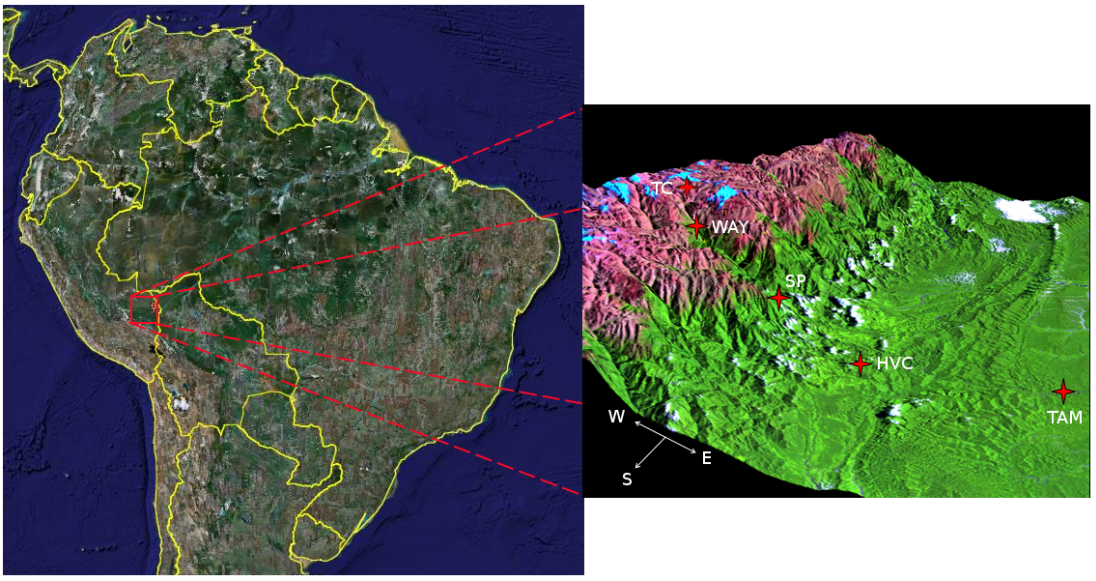

Figure 1.5: Location of the study sites of Andes Biodiversity and Ecosystems Research Group investigated as part of this thesis: Tambopata (TAM), Hacienda Villa Carmen (HVC), San Pedro (SP), Wayqecha (WAY) and Tres Cruces (TC). Site locations (TAM: 12°49′50″ S, 69°16′11″ W, HVC: 12°49′50″ S, 69°16′11″ W, SP: 13°02′56″ S, 71°32′13″ W, WAY: 13°11′24″ S, 71°35′13″ W and TC: 13°07′19″ S, 71°36′54″ W) are superimposed on a not-to-scale, northwest facing topographic representation of the eastern flank of the Andes (image: P. Zelazowski)

.

organic soils in montane settings may indicate that these soils operate differently to those of the lowlands.

We hypothesise that upland soils in the montane environments will represent sources or weaker sinks of atmospheric CH_4 than their lowland counterparts. Alternatively, these soils may follow general assumptions delineating soil source-sink activity and principally act as a sink for atmospheric CH_4.

2. To investigate the controls on soil-atmosphere CH_4 exchange. In this context we test controls on net CH_4 fluxes from these soils in terms of the expected biophysical constraints on the activity of high-affinity methanotrophs facilitating the uptake of atmospheric CH_4 and that of methanogenic and low-affinity methanotrophic communities that determine source-sink behaviour through their relative activity.

   We hypothesise that bulk soil O_2 concentration as a proximal measure of the balance between conditions suitable for methanogenic and low-affinity methanotrophic activity will best explain net CH_4 fluxes. Alternatively, if uptake dom-

inates soil-atmosphere CH_4 exchange in these environments proxies for constraints on the supply of CH_4 to high-affinity methanotrophic communities such as water filled pore-space will best explain net CH_4 fluxes.

3. To investigate the relationship between gross processes of CH_4 consumption and production. Variations in net CH_4 flux imply variations in either the rates of CH_4 uptake by high-affinity methanotrophic communities or the balance between the activity of methanogenic and low-affinity methanotrophic communities. In the the first instance, net CH_4 fluxes are determined by methanotrophic activity. However, in the second instance the balance between production and consumption reflects the capacity for methanogens and methanotrophs to exploit anoxic and oxic niches within these soils.

   We hypothesise that methanogenic processes will be active, despite oxic conditions, in these upland soils. We expect variations in soil CH_4 cycling to be driven by variations in production rather than consumption. In this respect, variations in production are expected to be limited by the availability of substrates whilst consumption is expected to be dependant on the supply of CH_4. Alternatively, consumption may control net uptake of CH_4 when in situ production is negligible or where zones of production and consumption are vertically stratified.

These aims are addressed in three data chapters relating to: lowland western Amazonian terra firme rainforest in Chapter 2, Andean premontane and montane forest in Chapter 3 and Andean puna grassland and wetland in Chapter 4. In Chapter 2, we focus on differences in soil-atmosphere CH_4 exchange and environmental drivers in two lowland terra firme forest soils of different porosity through intensive field measurement campaigns during wet and dry seasons. Furthermore, we conduct a laboratory incubation experiment to deconvolve gross rates of production and consumption under oxic conditions and investigate their distribution with depth in these soils. In Chapter 3, we focus on spatial and temporal variations in soil-atmosphere CH_4 exchange and environmental drivers among and within premontane, lower montane and upper montane forests through long-term monthly measurements. In Chapter 4, we focus on spatial and temporal variability in soil-atmosphere CH_4 exchange from humid puna upland

and wetland soils through long-term monthly measurements and intensive seasonal campaigns. Similarly to Chapter 2, we also conduct a laboratory incubation experiment to deconvolve gross rates of production and consumption under oxic conditions and investigate relationships between these activities across the landscape. Finally the findings of these chapters are synthesised in Chapter 5 where we attempt to assess the source-sink activity of the study region and discuss differences in the controls on soil-atmosphere CH_4 exchange and below-ground CH_4 cycling across the transect.

Chapter 2

Drivers of methane flux from two terra firme forest soils in the western Peruvian Amazon

Sam Jones[1], Marco Antonio Maldonado Zevallos[2], Karol Mejia Espinoza[2], Yit Arn Teh[3] and Patrick Meir[1]

[1]School of GeoSciences, University of Edinburgh, Edinburgh, United Kingdom, [2]Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru, [3]Institute of Biological and Environmental Sciences, University of Aberdeen, United Kingdom

This chapter reports soil-atmosphere CH_4 exchange and soil environmental conditions for lowland forest soils in the Western Amazon. Here we report data from two 9 day measurement campaigns conducted in December 2011 and July 2012 and a laboratory incubation experiment. Supplementary data can be found for the field measurements in Appendix B and the incubation experiment in Appendix A. Contributions to this chapter have been made by Sam Jones, Marco Antonio Maldonado Zevallos, Karol Mejia Espinoza, Patrick Meir and Yit Arn Teh. Yit Arn Teh and Patrick Meir provided advice in all stages of the work. Sam Jones was responsible for the sampling approach and measurements. Marco Antonio Maldonado Zevallos and Karol Mejia Espinoza provided assistance collecting field measurements during 2010 and 2011 campaigns, respectively. Sam Jones conducted all laboratory work, data analysis and writing.

2.1 Abstract

The tropics are poorly constrained in global atmospheric CH_4 budgets. This suggests that current source-sink inventories poorly characterise the activity of these landscapes. The soils of extensive tropical upland forests, which are typically generalised as a sink for atmospheric CH_4, exhibit significant spatial and temporal variability, as a function of soil texture and rainfall patterns, in their interaction with the atmosphere. In this context an improved understanding of the magnitude and dynamics of soil-atmosphere exchange in these environments may help to reconcile differences between bottom-up and top-down budget estimates.

We investigated soil CH_4 cycling in two terra firme forests, growing on ultisol and inceptisol soils, in the Tambopata-Candamo Reserve, Madre de Dios, Peru. Drivers of net CH_4 flux were investigated in two 9 day field campaigns during the wet season (2011) and dry season (2012). Gross consumption and production rates were deconvolved in these soils using a ^{13}C - CH_4 tracer approach in laboratory incubations. We aimed to provide information about the magnitude of net CH_4 fluxes for these soils and specifically hypothesized that variations would be driven by soil O_2 concentration and that methanogenesis would occur in these soils irrespective of oxic conditions.

These soils acted similarly to those of other Amazonian terra firme forests with mean net CH_4 fluxes and standard errors during wet and dry season of -1.39 (0.07) and -1.59 (0.06) for the ultisols and -0.41 (0.10) and -0.95 (0.06) mg C-CH_4 m^{-2} d^{-1} for the inceptisols, respectively. Whilst both soils acted as a sink for atmospheric CH_4 we observed a shift in the balance between consumption and production between seasons in the inceptisol soils. We reject our hypothesis, that O_2 concentration drives net flux, as no significant relationships were identified between flux rate and environmental parameters for the ultisol soils, whilst, the single strongest predictor in the inceptisol soils was water-filled pore space (WFPS). Deconvolving gross process rates suggest that methanogenesis is occurring in these soils despite oxic conditions. The difference between gross process rates, resulting from high methanotrophic activity, was greatest in the surficial mineral soils whilst processes were more finely balanced

at depth. We suggest that both methanotrophy and methanogenesis drive the seasonal shift observed in the inceptisol soils whilst methanotrophy is dominant in the ultisols. These differences between soil types support previous findings that spatial variations in the biophysical controls on both methanotrophy and methanogenesis are complex both within and between sites.

2.2 Introduction

Methane (CH_4) plays an important role in the atmosphere as a greenhouse gas and reactive agent in tropospheric and stratospheric chemistry (Cicerone and Oremland, 1988). In order to attribute past and predict future changes in the atmospheric concentration of CH_4 an understanding of how and why the strength of its sources and sinks vary spatially and temporally is required. Soils are significant in this respect as they act through microbial activity, to both produce and consume CH_4. The net exchange of a soil environment with the atmosphere as a source or sink is fundamentally determined by the balance between these processes (Le Mer and Roger, 2001). Soil CH_4 emissions represent the largest natural source to the atmosphere, accounting for 20 - 40 $\%$ of the contemporary global annual source budget and a greater proportion prior to the industrial revolution (Denman et al., 2007; Lelieveld et al., 1998). Such source activity is typically related to inundated wetland environments where production of CH_4 by methanogenic microbial communities represents the terminal step of organic matter breakdown after successive depletion of more energetically favourable electron acceptors such as oxygen (O_2), nitrate and sulphate below the water-table. Consumption processes also play an important role in such settings, with low-affinity aerobic methanotrophic bacteria exploiting the abundance of CH_4 and O_2 close to the interface between oxic vadose and anoxic saturated phases, acting to consume the majority of endogenously produced CH_4, and thus, reducing the strength of potential sources (Conrad, 1996; Segers, 1998; Von Fischer and Hedin, 2007). Similarly, soils play an important role, accounting for ~ 6 $\%$ of the contemporary global annual sink budget, as the largest biological sink for atmospheric CH_4 (Denman et al., 2007). Such uptake is typically related to upland soils where oxic conditions limit production and high-

affinity methanotrophic bacteria exploit CH_4 at concentrations close to atmospheric levels (Bender and Conrad, 1992; Conrad, 1996).

Changes in precipitation and wetland extent, particularly in the tropics, have been used to explain global variations in atmospheric CH_4 concentration observed from ice-core records extending into the late Pleistocene (Loulergue et al., 2008; Fischer et al., 2008; Singarayer et al., 2011) and in direct atmospheric measurements since the mid-20th century (Mikaloff Fletcher et al., 2004a; Bousquet et al., 2006; Chen and Prinn, 2006; Dlugokencky et al., 2009). Despite this the tropics are poorly characterized when top-down measurements of atmospheric CH_4 concentration and inverse modelling simulations are compared with bottom-up estimates of source-sink budgets (Mikaloff Fletcher et al., 2004b; Frankenberg et al., 2005, 2008). This is particularly true of tropical South America where source-sink inventories and process models tend to underestimate regional observations of atmospheric CH_4 concentration (Melack et al., 2004; Mikaloff Fletcher et al., 2004b; Bloom et al., 2010b). In this respect, evidence of unattributed sources in humid upland tropical forests, which cover ~ 35 $\%$ of the continent, and observations that wet upland or 'transitional' soils, close to saturation, can vary between uptake and emission in the absence of extensive water-table development suggest that an improved understanding of CH_4 cycling in such environments might help to explain regional discrepancies (Eva et al., 2004; do Carmo et al., 2006; Miller et al., 2007; Spahni et al., 2011).

The majority of the world's humid upland tropical forests are found in South America (Achard et al., 2002). Globally these environments have been estimated, based on limited field observations, to account for 10 - 20 $\%$ of the soil sink for atmospheric CH_4 (Potter et al., 1996; Dutaur and Verchot, 2007). However, net CH_4 uptake by the soils of these forests varies considerably with soil texture and moisture. For example, seminal studies by Steudler et al. (1996) and Keller et al. (1986) reported mean annual net CH_4 flux rates for eastern Amazonian upland forest on coarse textured ultisols and fine grained oxisols of -1.3 and -0.3 mg C-CH_4 m^{-2} d^{-1}, respectively. Similarly, net flux rates reported by Verchot et al. (2000) and Keller et al. (2005), respectively for two eastern Amazonian oxisols, are lower in the dry season with means of -0.74 (0.16)

and -0.2 (0.2) mg CH_4 m^{-2} d^{-1} than in the wet season with means of 0.02 (0.16) and -0.1 (0.2) mg CH_4 m^{-2} d^{-1}. In this context, evidence of production within soil profiles and transient emissions are common, particularly, in finer textured soils (Keller et al., 1986; Verchot et al., 2000; Davidson et al., 2004). Such variations in net flux reflect diffusional constraints, imposed by changes in the extent and connectivity of water-filled pore space (WFPS), on gas transport and substrate availability to methanotrophic and methanogenic microbes (Conrad, 1996; Smith et al., 2003; Von Fischer and Hedin, 2007).

In contrast to the vertical stratification of aerobic and anaerobic processes imposed by the water-table in wetland soils, production in upland soils indicates spatially heterogeneous environments where methanogenesis occurs in anoxic micro-sites within an oxic matrix that supports aerobic high-affinity methanotrophy (Sexstone et al., 1985; Smith et al., 2003; Teh et al., 2005). In soils like this, oxygen (O_2) availability is high, and methanotrophy may be more strongly limited by CH_4 availability to high-affinity communities; for example, soil gas profile measurements in upland tropical forest soils typically indicate the bulk soil matrix is oxic (10 - 21 $\%$ O_2) whereas CH_4 concentrations are sub-ambient (< 1.8 ppm CH_4) (Bender and Conrad, 1992; Verchot et al., 2000; Cleveland et al., 2010). Similarly, methanogenesis is limited by the extent of micro-niches with favourable redox conditions, which is controlled by the balance between rates of inward diffusion of O_2 from the atmosphere and O_2 consumption by aerobic respiration (Conrad, 1996; Smith et al., 2003; Von Fischer and Hedin, 2007). Work by Verchot et al. (2000) support such mechanisms, suggesting that positive relationships between net CH_4 flux rate and both WFPS and carbon dioxide (CO_2) emissions across an upland deforestation chronosequence in eastern Amazonia represents diffusional constraints on CH_4 supply to methanotrophs in drier sites and the promotion of methanogenesis in wetter sites where biological O_2 demand is high. Despite this, studies relating net CH_4 flux and below-ground CH_4 cycling processes to O_2 concentrations in the tropics have previously focused on highland environments where availability of dissolved and free O_2 has been shown to vary on time periods of days (Silver et al., 1999; Teh et al., 2005; Liptzin et al., 2011).

Here we report net CH_4 fluxes from two upland tropical forests in the Western Amazon on ultisol and inceptisol soils during the wet season and dry season of 2011-2012. We conducted daily measurements of net CH_4 flux and environmental parameters over a 9 day period in each season to investigate drivers of soil-atmosphere CH_4 exchange from these soils. Lastly, we investigated gross methanotrophic and methanogenic activity from incubations of soils sampled at different depths throughout the soil profile. We aim to provide information about the magnitude of dry and wet season net CH_4 fluxes for these soils and investigate the relationship between seasonal variations in net flux and environmental conditions. We specifically hypothesise that 1) variations in net CH_4 flux are explained by soil O_2 concentration as it represents an integrated measure of diffusional constraints imposed by WFPS and uptake of O_2 by aerobic respiration and 2) methanogenesis occurs in these soils despite oxic conditions in the bulk soil environment.

2.3 Materials and methods

2.3.1 Study sites

Two sites, hereafter referred to by soil type, ~ 2 km apart were selected for study in the Tambopata-Candamo Reserve, department of Madre de Dios, Peru. The sites are adjacent to long term RAINFOR plots TAM-09 (N -12.83 S -69.28, 197 m asl) and TAM-05 (N -12.83 S -69.27, 220 m asl), respectively. These sites were chosen as they represent moist, mixed old growth terra firme forest experiencing the same climatic conditions but with differing soil histories (Asner et al., 2013). The first site occurs on a late Holocene floodplain with inceptisol soils, whilst the second is characterised by ultisol soils on a Pleistocene terrace. Air temperature is relatively aseasonal with an annual mean of 26 $^{\circ}\mathrm{C}$. In contrast, precipitation is strongly seasonal with more than 75 $\%$ of the annual mean of 2600 mm falling in the wet season between September and March (Zimmermann et al., 2009).

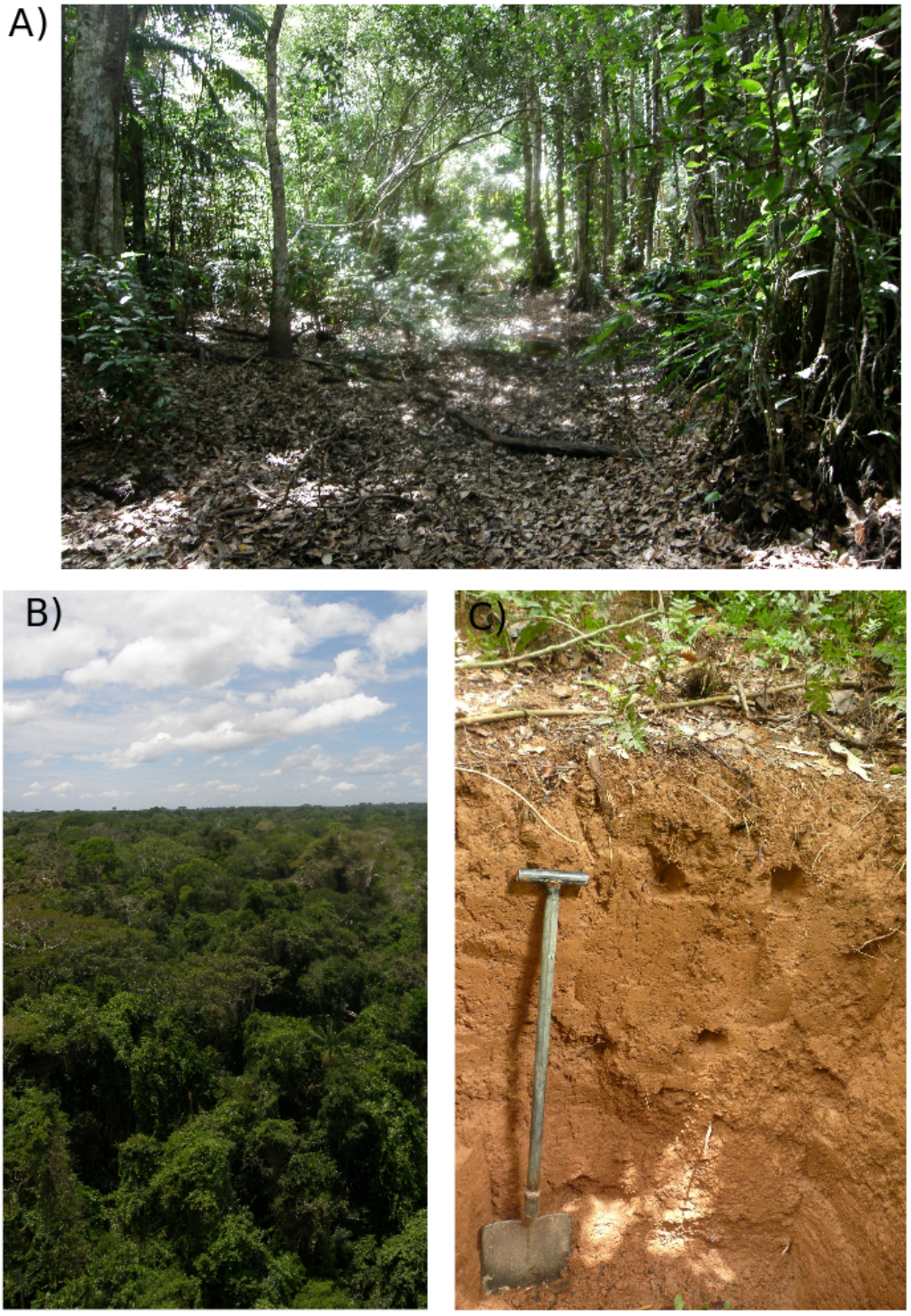

Figure 2.1: Tambopata study area: a) an example of forest structure within TAM-09, b) an overview of the forest canopy at Tambopata from a ~ 40 m tower and c) a soil pit showing profile of ultisol soils found at TAM-05.

2.3.2 Field sampling

Two plots were selected at each site for field sampling campaigns conducted over 9 consecutive days during wet (01/12/2011 - 09/12/2011) and dry season (15/07/2012 - 23/07/2012). The plots at each site, approximately 20 m apart, were selected on the basis of their proximity and broad similarity to the adjacent RAINFOR plot. Each plots was ~ 20 by 20 m and before each campaign instrumented at 6 locations 5 - 10 m apart. The position of sampling equipment tended to be positioned between trees as dictated by the presence of large tree roots. Sampling equipment was removed between campaigns to prevent loss and reinstalled in the same plots but at different locations. At each location, a soil collar and three soil gas equilibration chambers were installed approximately four weeks prior to each campaign to minimise the influence of disturbances (Varner et al., 2003). Sampling locations had a footprint of ~ 0.5 m^2. During measurement campaigns each sampling location was visited daily between the hours of 08:00 and 16:00 and the day to day order of visits varied to minimise effects of temporal variability associated with sunrise and sunset at ~ 06:00 and 18:00. Soils were sampled from beneath 3 collars in each plot at the end of the wet season campaign to provide material to characterize soil properties and conduct incubation experiments.

2.3.2.1 Flux measurements

Soil-atmosphere CH_4 and CO_2 fluxes were determined daily at each location using a static chamber approach (Livingston and Hutchinston, 1995). Measurements were initiated by gently sealing, with a section of inner tube, cylindrical caps to 20 cm diameter collars to create a chamber of ~ 0.08 m^3 over a soil surface of ~ 0.03 m^2. Collars were inserted to a depth of ~ 5 cm. Caps were equipped with a gas sampling port, air pressure equilibration port, and a 9 V computer fan (Pumpanen et al., 2004). Ambient air temperature at 5 cm above the surface, chamber air temperature and atmospheric pressure were measured and a 20 ml gas sample taken at 4 discrete time-steps over a period of ~ 20 minutes following the initiation of a measurement. Gases were sampled using a stopcock and 60 ml gas tight syringe and were stored in over-pressured,

pre-evacuated 12 ml Exetainers (Labco Ltd., UK). Temperatures and atmospheric pressure were measured using a type-k thermocouple (Omega Engineering Ltd.,UK.) and a Garmin GPSmap 60CSx (Garmin Ltd.,USA). Subsequently, CH_4 and CO_2 concentrations were determined by gas chromatography. Amounts, in moles, were calculated from concentration, chamber volume, chamber temperature and atmospheric pressure following ideal gas law.

Fluxes, in mol m^{-2} d^{-1}, were calculated in R (R Core Team, 2013) using the HMR package (Pedersen, 2012). Following the criteria outlined by Pedersen et al. (2010), non-linear HMR or linear models were fitted to time-series of amount in chamber headspaces. Significance was determined at the $p < 0.05$ level with emission and uptake indicated by positive and negative flux values, respectively. A detection limit for each flux is calculated from the regression coefficients estimated in Parkin et al. (2012) appropriate to the model fitted at a measurement precision for CH_4 and CO_2 amount, respectively, of 2.6 and 1.3 % (CV of air, $n > 30$). Significant fluxes below detection limits were reported to minimise bias (Gilbert, 1987). Non-significant fluxes below detection limits were deemed to be net zero fluxes (i.e. reported as 'no flux'), whilst those greater than detection limits were considered to have resulted from failures during sampling, storage or analysis (i.e. reported as 'NA'). In both cases these values were excluded from statistical analysis.

2.3.2.2 Soil gas profiles

Soil gas equilibration chambers were vertically buried at 10, 30 and 50 cm at each location (Silver et al., 1999; Teh et al., 2005). Chambers had an internal volume of 50 ml and a surface area of 57 cm^2. Each consisted of a length of gas-permeable silicone rubber tubing (AP202/60 - 35 mm inner diameter x 1.5 mm wall, Advanced Polymers Ltd., UK) sealed at one end with silicone cement and the other with a butyl rubber bung (Figure 2.2 a). A suitable length of tygon tubing was passed through a hole in the bung and capped with a stopcock to allow sampling at the surface. Chambers were encased in plastic mesh to protect the membrane during installation. Chambers were installed by coring a 3.5 cm diameter hole to the required soil depth, inserting the

chamber and then back filling with the removed soil so that the stopcock emerged at the surface (Figure 2.2 b). Typical of similar designs, soil gas equilibration chambers were capable of equilibrating with the external atmosphere in less than 24 hours (Holter, 1990; Jacinthe and Dick, 1996; Kammann et al., 2001).

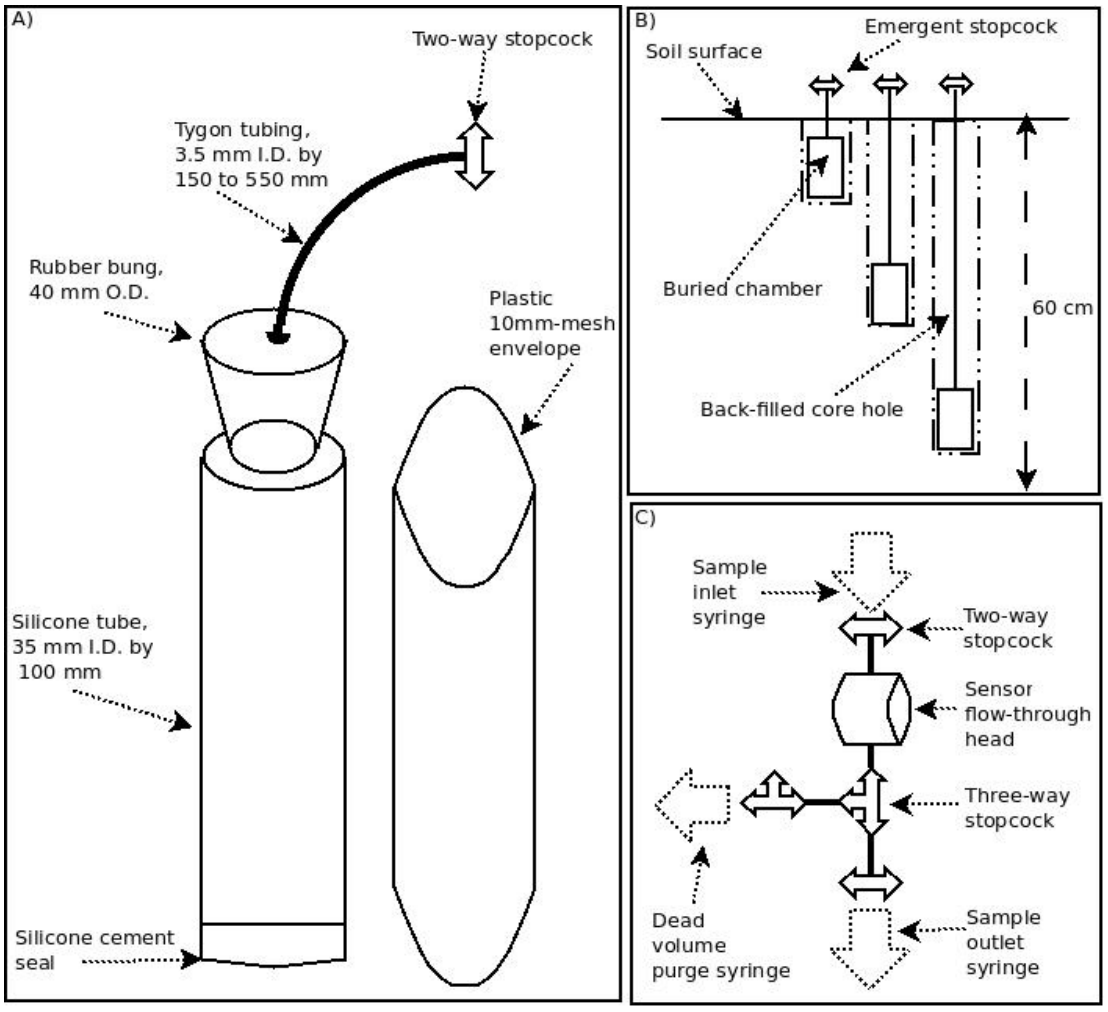

Figure 2.2: Soil gas equilibration chambers: a) outline of soil gas equilibration chamber construction, b) outline of soil gas equilibration chamber field installation and c) outline of O_2 sensor set-up.

Soil O_2 concentrations were determined daily at each location and depth. Measurements were made by withdrawing 40 ml of gas from a chamber using a stopcock and gas tight syringe. Prior to gas sampling, dead volumes within the sampling apparatus were evacuated to minimise contamination by residual atmospheric air (Figure 2.2 c). The gas sample was then collected and passed through the flow-through head of an MO-200 O_2 sensor (Apogee Instruments Inc., USA) into a second syringe. Following collection of the O_2 reading, soil gas was re-injected back into the soil using

the second syringe. The exception was the final measurement collected at the end of each campaign, which rather than being re-injected was stored in an over-pressured, pre-evacuated 12 ml Exetainer (Labco Ltd., UK) for determination of CH_4 and CO_2 concentration by gas chromatography. The O_2 sensor was calibrated in the field with air, as required, prior to measurements. The concentration, in percent, was recorded with a precision of 0.1 $\%$.

2.3.2.3 Soil water content and soil temperature

Soil water content and soil temperature were measured daily within the sampling footprint of each location. Soil temperature was measured at 10 cm using a type-k penetration probe (Omega Engineering Ltd.,UK). Percentage soil water content, integrated over the upper 20 cm of soil, was measured using a CS620 Hydrosense unit equipped with 20 cm rods (Campbell Scientific Inc, USA). WFPS was calculated from percentage water content and plot estimates of porosity.

2.3.2.4 Soil sampling

At the end of the wet season campaign half the locations in each plot were sampled for soil. A 60 cm deep pit was dug adjacent to collars in chosen locations and paired 100 ml volumetric and ~ 200 g bulk soil samples taken. Volumetric samples, used to determine bulk density, were taken by inserting density rings with a diameter of 5 cm and a depth of 5.1 cm into pit sidewalls at 2.5, 10, 30 and 50 cm below the soil surface. Bulk samples consisted of material removed from between 0 - 5, 5 - 15, 25 - 35 and 45 - 55 cm depth. Prior to use in incubations and determination of soil properties, bulk samples were gently homogenized and large root fragments removed by hand. In both the determination of soil properties and incubations each of the 3 replicates per depth per plot were used.

2.3.3 Laboratory-based ^{13}C isotope tracer studies

Approximately 100 g of soil, at field water content, from each of the sampled locations and depths, were placed in 1 L Kilner jars. Blanks consisted of jars containing no soil. Jars were loosely sealed with screw-cap lids fitted with septa ~ 48 hours before the start of the experiment. To initiate incubations, jars were vented and fully sealed before being spiked with 1 ml of N_2 carrier with SF_6 and $^{13}CH_4$ concentrations of ~ 0.3 and 70 μL L^{-1}, respectively. Jar headspace was mixed with a 60 ml syringe and pre-incubated for 30 - 60 minutes to allow the tracers to fully equilibrate within the headspace (Von Fischer and Hedin, 2002). Following the pre-incubation period, 100 ml of N_2 was injected and the headspace mixed at 4 discrete times over the course of 10 - 12 hours. The resulting overpressure was sampled and stored in evacuated 60 ml Wheaton bottles sealed with 20-mm butyl septa (Geo-microbial Technologies Inc., USA). Five millilitre sub-samples were taken from each bottle for analysis by gas chromatography, with measurements corrected for sampling dilution, and the remainder analysed by isotope ratio mass spectrometry. Incubations took place in the dark at 24°C reflecting typical temperatures for this study area in the range of 21.5 to 26.5°C (Malhi et al., 2013). Incubations were leak checked based on changes in the concentration of SF_6. Headspace volume was calculated based on oven dry soil weight, gravimetric water content and particle density.

2.3.3.1 Isotope pool dilution model

The ^{13}C-tracer isotope pool dilution model developed and the assumptions described by Von Fischer and Hedin (2002) was used to deconvolve the contribution of gross consumption and production of CH_4 to the net change in amount of CH_4 in incubation headspace. Following Michaelis-Menten kinetics and with the assumptions that CH_4 concentration is below K_m (Bender and Conrad, 1992) and that production contributes negligible amounts of $^{13}CH_4$ over the course of the incubation period, the rate of consumption can be determined from the first order decay of the amount of $^{13}CH_4$ (Von Fischer and Hedin, 2002), described by,

$$[^{13}CH_4]_t = [^{13}CH_4]_0 \exp^{-k_{13}t} \tag{2.1}$$

where $[^{13}CH_4]_t$ is the concentration of $^{13}CH_4$ at a given time, $[^{13}CH_4]_0$ is the concentration of $^{13}CH_4$ at time zero, k_{13} is the first order decay rate for the consumption of $^{13}CH_4$ and t is time since the beginning of the incubation.

The balance between the rate of production and consumption is determined from relationships between amount of CH_4 and the atom percent of $^{13}CH_4$ against time and each other. When consumption is greater than production, the amount of CH_4 decreases with time and a positive relationship is observed between amount of CH_4 and atom percent of $^{13}CH_4$ as $^{12}CH_4$ is preferentially consumed with respect to $^{13}CH_4$. When production is greater than consumption, the amount of CH_4 increases with time and a negative relationship is observed between amount of CH_4 and atom percent of $^{13}CH_4$ as the headspace is diluted by isotopically light CH_4. When process rates are equal the amount of CH_4 will remain constant with respect to time and the atom percent of $^{13}CH_4$ will decrease from time zero at a constant concentration of CH_4. The change in amount of CH_4 is described by,

$$[CH_4]_t = \frac{P}{k_{12}} - \left(\frac{P}{k_{12}} - [CH_4]_0\right) \exp^{-k_{12}t} \tag{2.2}$$

where $[CH_4]_t$ is the concentration of CH_4 in the system at a given time, P is the gross rate of CH_4 production, k_{12} is the first order decay rate for $^{12}CH_4$ and $[CH_4]_0$ is the concentration of CH_4 at time zero. The change in the ratio of the amount of $^{13}CH_4$ to CH_4 follows as,

$$\frac{[^{13}CH_4]_t}{[CH_4]_t} = \frac{[^{13}CH_4]_0 \exp^{-k_{13}t}}{\frac{P}{k_{12}} - \left(\frac{P}{k_{12}} - [CH_4]_0\right) \exp^{-k_{12}t}} \tag{2.3}$$

and,

$$AP_t = \frac{[^{13}CH_4]_t}{[CH_4]_t} + AP_p \tag{2.4}$$

where AP_t is the atom percent of $^{13}CH_4$ at a given time and AP_p is the atom percent of produced CH_4.

2.3.3.2 Determination of gross process rates

The rate of CH_4 consumption is calculated as;

$$C = bvk_{12} \tag{2.5}$$

where C is the rate of CH_4 consumption, b is the concentration of CH_4 at the soil surface and v is the volume of the headspace. A value of 1.8 µL L^{-1} is used for b to standardise rates of C to atmospheric CH_4 concentration (Von Fischer and Hedin, 2002). k_{13} is estimated from the linear regression of the natural log of the concentration of $^{13}CH_4$ against time (equation 2.1) and k_{12} is then calculated as,

$$k_{12} = k_{13}\frac{1}{\alpha} \tag{2.6}$$

where α is the fractionation factor for methane consumption. A value of 0.98 was adopted for α with conceivable variations unlikely to introduce large biases (Von Fischer and Hedin, 2002).

The rate of CH_4 production, P, is estimated by simultaneously fitting equations 2.2 and 2.3 to observations of $^{13}CH_4$ and CH_4 concentration. P is calculated recursively to optimise the best solution via minimisation of the normalised total error between observed and predicted values. Normalised total error,E, is calculated as;

$$E = \left(\sum_{t=1}^{n} \frac{|AP_{obs}(t) - AP_{pred}(t)|}{SD_{AP_{obs}}}\right) N_{AP} + \left(\sum_{t=1}^{n} \frac{|[CH_4]_{obs}(t) - [CH_4]_{pred}(t)|}{SD_{[CH_4]_{obs}}}\right) N_{[CH_4]}$$

(2.7)

With normalisation factors for isotope data, N_{AP}, and concentration data $N_{[CH_4]}$ calculated as,

$$N_{AP} = \frac{SD_{AP_{obs}}}{SD_{AP_{prec}}} \tag{2.8}$$

and,

$$N_{[CH_4]} = \frac{SD_{[CH_4]_{obs}}}{SD_{[CH_4]_{prec}}} \tag{2.9}$$

where $SD_{AP_{obs}}$ and $SD_{[CH_4]_{obs}}$ are the standard deviations of observations and $SD_{AP_{prec}}$ and $SD_{[CH_4]_{prec}}$ is the analytical precision for mass spectrometry and gas chromatography measurements.

Recursive optimization was implemented using the BB package (Varadhan and Gilbert, 2014) in R (R Core Team, 2013) with a starting condition and lower limit for P of 0 µL L^{-1} s^{-1}. The net CH_4 flux rate, F, is calculated as the difference between P and C,

$$F = P - C \tag{2.10}$$

2.3.4 Laboratory analyses

2.3.4.1 Gas chromatography

Gas samples were analysed by gas chromatography on a Thermo TRACE GC Ultra (Thermo Fisher Scientific Inc., USA) with a N_2 carrier gas. A flame ionization detector (FID), methanizer-FID and electron capture device (ECD) were used to determine CH_4, CO_2 and SF_6 concentrations, respectively. Analytes were separated using a Hayesep

Q 100/200 column. The instrument was equipped with a 2 ml sample loop and oven temperature was 60°C. Detector responses were calibrated using three or more, triplicated, certified gas standards (CK Gas Products Ltd., UK) and a coefficient of variance < 5 % was calculated for all detectors. Samples for all field flux measurements were introduced to the sample loop using a custom-built autosampler (University of York, UK); whilst those from soil gas equilibration chambers and incubations were manually injected with a 10 ml, low dead volume gas-tight syringe (VICI Precision Sampling, USA).

2.3.4.2 Isotope Ratio Mass spectrometry

The isotopic composition of C-CH_4 in incubation gas samples was analysed by mass spectrometry on a Finnigan Deltaplus XP GC-IRMS coupled to a Gasbench II and an automated trace gas PreCon (Thermo Fisher Scientific Inc., USA) at University of St Andrews, UK. Sample bottles were flushed into the PreCon over a period of 700 s by a stream of helium flowing at rate of 0.4 ml s^{-1}. The gas stream passes through a chemical trap, containing magnesium perchlorate to remove water vapour and Carbosorb to remove CO_2, into a liquid nitrogen cryotrap which removes residual condensible gases. Non-condensible gases then pass into an oven, at 950°C with a nickel-platinum catalyst, where CH_4 is oxidised to CO_2 and subsequently pre-concentrated in a second liquid nitrogen trap over a period of 320 s. CO_2 is then cryofocused in a third trap and injected into the IRMS via the Gasbench. System linearity and precision across the sample concentration range was confirmed with an in-house methane standard with a δ ^{13}C value of -48.2 ‰ relative to VPBD and a coefficient of variance over the analysis period of 0.06 %.

2.3.4.3 Soil physical properties

Oven dry mass and water content of soil and litter samples was determined after 24 hours at 105°C. Bulk density was calculated using density ring samples as oven dry mass per soil field volume. Particle density was determined using 10 ml pycnometers

and the methodology described in Klute et al. (1986). Porosity was calculated as the difference between 1 and the fraction of bulk and particle density.

2.3.4.4 Soil chemical properties

Following Sparks et al. (1996), soil pH was determined using a HANNA pHep 4 tester (HANNA Instruments, USA), with a precision of 0.1 pH, in a slurry with a 1:1 ratio of air dried soil to deionised water. Percent C and N were determined using a Costech ECS4010 elemental analyser elemental analyser with a zero-blank autosampler (Costech Analytical Technology Inc., USA) coupled to a Finnigan Deltaplus XP GC-IRMS. Air dried soil was finely ground and 10 - 20 mg aliquots weighed out into tin capsules. Capsules were crimped, balled and weighed with a precision of 0.001 mg. Detector response was calibrated using triplicates of empty tin capsules as blanks and a certified soil standard with 1.65 $\%$ C and 0.14 $\%$ N (Elemental Microanalysis Ltd., UK), prepared in the same manner as samples, at three content levels. Coefficients of variance for C and N were < 1.5 $\%$ and 3.5 $\%$, respectively.

2.3.5 Statistical analysis

Statistical analysis was conducted in R and significance, unless stated otherwise, reported at $p < 0.05$ (R Core Team, 2013). Reported daily and seasonal means of gas fluxes and environmental conditions are simple plot averages of day time measurements. Environmental parameters were not typically normally distributed. This pattern was carried through to the residuals of parametric statistical methods in many cases despite attempts at normalisation through typical means such as log-transformation. As such, relationships between daily measurements of fluxes and environmental conditions were investigated using the non-parametric Spearman's rank correlation coefficient. The residuals of linear model fits between CH_4 and WFPS and CO_2 fluxes among soil type and season met the assumptions of normality and homoscedasticity and are reported as such. A considerable number of CH_4 fluxes, with rates close to zero, were excluded from analysis as these values were indeterminable given method-

ological detection limits. Relationships between soil CH_4 concentration and soil O_2 and CO_2 concentrations were investigated using multiple linear regressions as residuals met assumptions of normality and homoscedasticity.

2.4 Results

2.4.1 Soil properties

Physical soil properties for each site are summarised in Table 2.1. The inceptisol soils have a higher mean bulk density than the ultisol soils. Within sites, mean bulk density (standard eror) increases with depth, from 1.20 (0.04) to 1.35 (0.05) g cm^{-3} in the inceptisol and 1.00 (0.03) to 1.23 (0.02) g cm^{-3} in the ultisol, between the 0 - 5 and 45 - 55 cm sampling layers. In contrast, mean particle density is similar in both sites, increasing with depth from 2.61 (0.02) and 2.60 (0.01) g cm^{-3} at 0 - 5 cm to 2.73 (0.00) and 2.73 (0.01) g cm^{-3} at 45 - 55 cm in the inceptisol and ultisol, respectively. As such, porosity is lower in the more compact inceptisol soils, ranging from 0.54 (0.01) to 0.51 (0.02), than in the ultisol, ranging from 0.63 (0.01) to 0.55 (0.01), and generally decreases with depth.

Table 2.1: Physical properties for inceptisol soils at site 1 and ultisol soils at site 2. Values are mean (standard error) for each site. n = 6 per site and depth.

Soil property	Depth (cm)	Inceptisol	Ultisol
Bulk density g cm^{-3}	0 - 5	1.20 (0.04)	1.00 (0.03)
	5 - 15	1.27 (0.03)	0.99 (0.04)
	25 - 35	1.33 (0.02)	1.18 (0.02)
	45 - 55	1.35 (0.05)	1.23 (0.02)
Particle density g cm^{-3}	0 - 5	2.61 (0.02)	2.60 (0.01)
	5 - 15	2.66 (0.01)	2.66 (0.01)
	25 - 35	2.72 (0.01)	2.71 (0.00)
	45 - 55	2.73 (0.00)	2.73 (0.01)
Porosity -	0 - 5	0.54 (0.01)	0.62 (0.01)
	5 - 15	0.52 (0.01)	0.63 (0.01)
	25 - 35	0.51 (0.01)	0.56 (0.01)
	45 - 55	0.51 (0.02)	0.55 (0.01)

Chemical soil properties for each site are summarised in Table 2.2. The mean C content

of the inceptisol soils is lower than that of the ultisol soils and, respectively, decreases with depth from 2.42 (0.28) to 0.32 (0.01) % C and 5.74 (0.58) to 0.97 (0.06) % C between the 0 - 5 and 45 - 55 cm sampling layers. Similarly, C:N ratios decrease between 0 - 5 and 45 - 55 cm from 8.69 (0.62) to 4.60 (0.38) in the inceptisol and 11.63 (0.43) to 8.64 (0.33) in the ultisol. Conversely, pH is higher in the inceptisol than the ultisol and increases with depth, respectively, from 3.9 (0.2) to 4.6 (0.0) and 3.5 (0.1) to 4.2 (0.0) between 0 - 5 and 45 - 55 cm.

Table 2.2: Chemical properties for inceptisol soils at site 1 and ultisol soils at site 2. Values are mean (standard error) for each site. n = 6 per site and depth.

Soil property	Depth (cm)	Inceptisol	Ultisol
pH -	0 - 5	3.9 (0.2)	3.5 (0.1)
	5 - 15	4.0 (0.1)	3.5 (0.0)
	25 - 35	4.4 (0.0)	4.1 (0.0)
	45 - 55	4.6 (0.0)	4.2 (0.0)
C %	0 - 5	2.42 (0.28)	5.74 (0.58)
	5 - 15	0.95 (0.05)	2.24 (0.11)
	25 - 35	0.45 (0.03)	1.20 (0.05)
	45 - 55	0.32 (0.01)	0.97 (0.06)
C:N -	0 - 5	8.69 (0.62)	11.63 (0.43)
	5 - 15	6.55 (0.23)	9.25 (0.12)
	25 - 35	5.24 (0.33)	9.92 (0.25)
	45 - 55	4.60 (0.38)	8.64 (0.33)

2.4.2 Field measurements

2.4.2.1 WFPS, air temperature and soil temperature

The inceptisol soils were wetter than the ultisol soils (Table 2.3). During dry and wet season, respectively, mean WFPS was 39.6 (0.5) and 63.3 (0.2) % in the inceptisol soils and 30.6 (0.5) and 43.2 (0.9) % in the ultisol soils. In both wet and dry season day to day variations in WFPS were similar between sites (Figure 2.3). During the wet season, WFPS peaked three times and increased by ~ 20 % over the sampling period. This between day variability was greater than variations between sampling locations within sites. During the dry season, day to day variations in WFPS were smaller and showed a slight decline over the sampling period. Temporal and spatial variations were

more similar during the dry than wet season.

Table 2.3: Campaign means for daily measurements during wet and dry season. Values are mean and (standard error). n = 9 per site and season.

Season	Site	Inceptisol	Ultisol
dry	Net CH_4 flux	-0.95 (0.06)	-1.56 (0.06)
wet	(mg C-CH_4 m^{-2} d^{-1})	-0.41 (0.10)	-1.39 (0.07)
dry	Net CO_2 flux	2.90 (0.09)	2.50 (0.08)
wet	(g C-CO_2 m^{-2} d^{-1})	4.28 (0.15)	5.15 (0.15)
dry	WFPS	39.6 (0.5)	30.6 (0.5)
wet	(%)	63.3 (0.9)	43.2 (0.9)
dry	O_2 concentration	20.5 (0.1)	20.8 (0.0)
wet	(% at 10 cm)	18.1 (0.1)	17.9 (0.1)
dry	O_2 concentration	20.2 (0.1)	20.4 (0.0)
wet	(% at 30 cm)	15.9 (0.2)	17.2 (0.1)
dry	O_2 concentration	19.8 (0.1)	20.1 (0.1)
wet	(% at 50 cm)	15.8 (0.2)	17.0 (0.1)
dry	Air temperature	21.5 (0.2)	22.8 (0.3)
wet	(°C)	27.7 (0.2)	28.2 (0.2)
dry	Soil temperature	20.2 (0.1)	20.5 (0.1)
wet	(°C)	25.6 (0.1)	25.5 (0.1)

Air and soil temperature were similar between sites (Table 2.3). During the dry season, air and soil temperatures were 21.5 (0.2) and 20.2 (0.1) °C at the inceptisol site and 22.8 (0.3) and 20.5 (0.1) °C at the ultisol site, respectively. Temperatures were higher in the wet season, with means for air and soil of 27.7 (0.2) and 25.6 (0.1) °C at the inceptisol site and 28.2 (0.2) and 25.5 (0.1) °C at the ultisol site. During both seasons, air and soil temperature were highly variable between days when compared with between and within site variability. Within days, temperature was related to time of sampling and was higher in the afternoon than morning.

2.4.2.2 Soil gas concentrations

Bulk soil atmosphere O_2 concentrations were typically lower in the inceptisol than the ultisol and decreased with depth (Table 2.3). O_2 concentrations were lower in the wet season than the dry season (Figure 2.4). During the dry season, mean O_2 concentrations decreased from 20.5 (0.1) and 20.8 (0.0) % at 10 cm depth to 19.8 (0.1) and 20.1 (0.1) % at 50 cm in the inceptisol and ultisol, respectively. Similarly during the wet season,

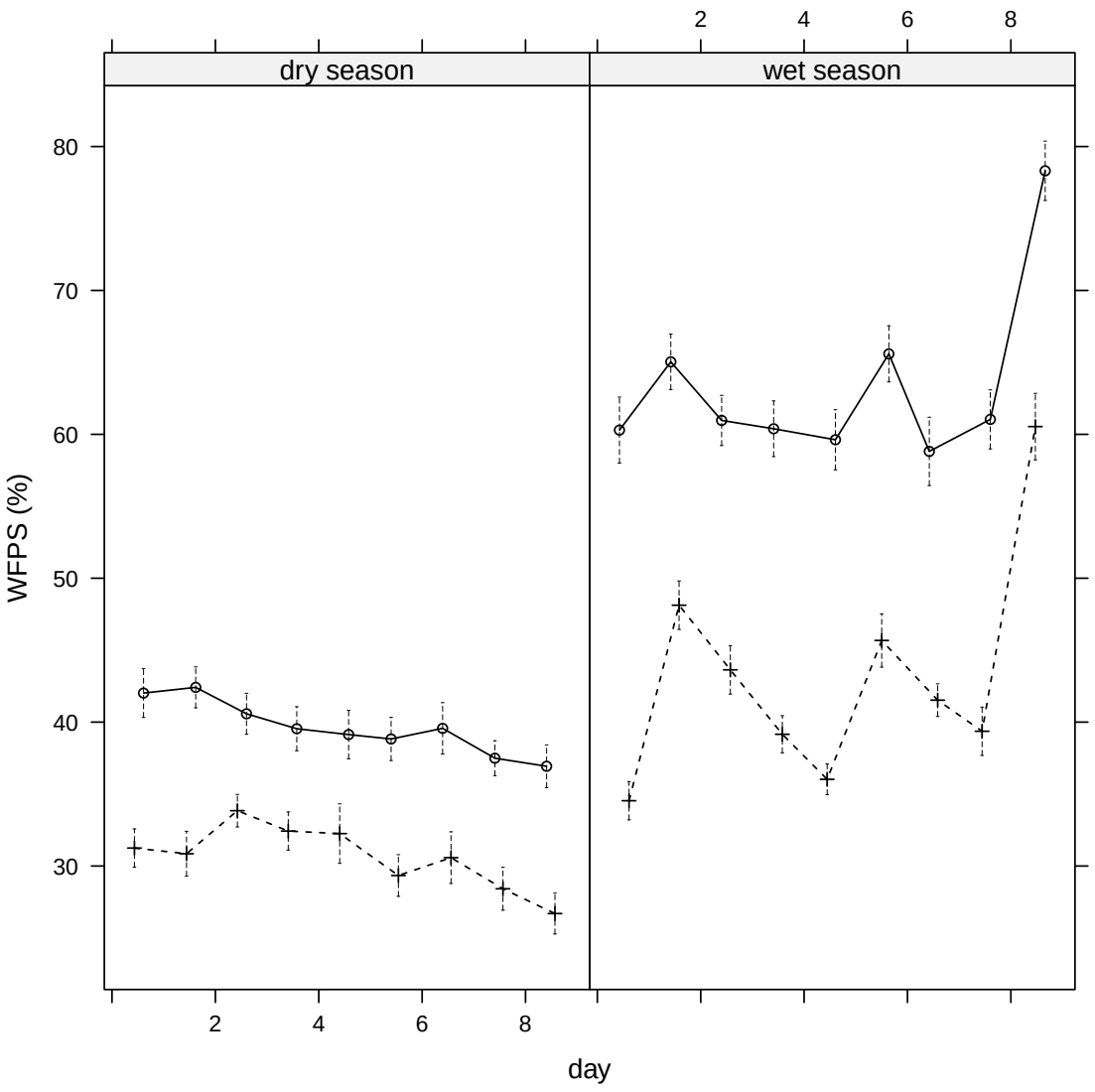

Figure 2.3: Daily averaged WFPS for both sites during dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars are standard errors (n = 12).

mean O_2 concentrations in the inceptisol and ultisol, respectively, decreased from 18.1 (0.1) and 17.9 (0.1) $\%$ at 10 cm depth to 15.8 (0.2) and 17.0 (0.1) $\%$ at 50 cm.

Soil CO_2 concentrations were higher in the inceptisols than the ultisols and typically increased with depth (Figure 2.5) . Within sites, CO_2 concentrations were higher in the wet than the dry season. Similarly, soil CH_4 concentrations were higher in the inceptisols and wet season than the dry season and ultisols (Figure 2.6). Soil CH_4 concentrations were typically sub-atmospheric, only exceeding 1.8 ppm in the inceptisol soils during the wet season. Wet season CH_4 soil concentrations in the inceptisol are also notably more variable than those in the ultisols or the dry season. Mean CH_4 con-

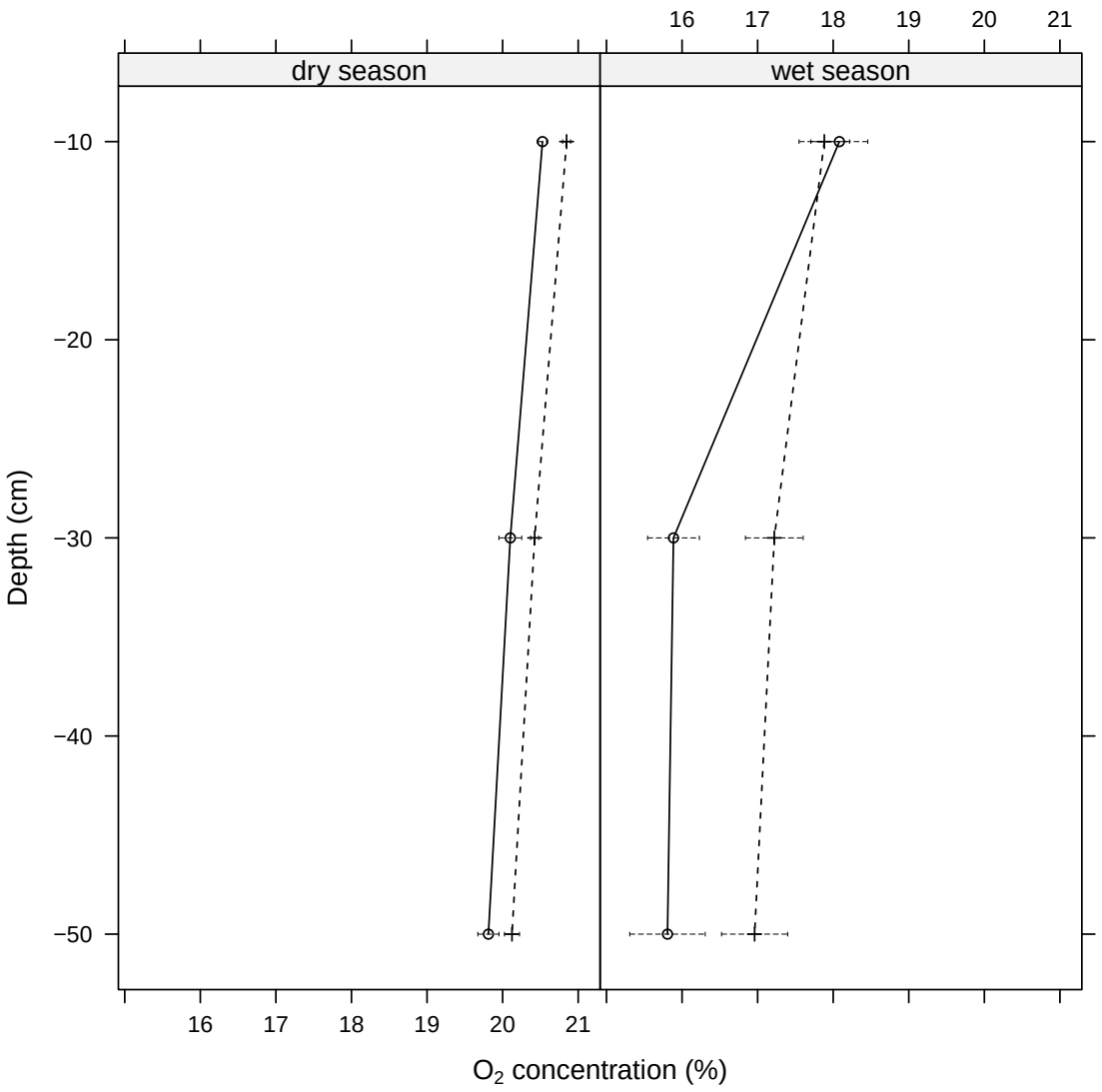

Figure 2.4: Depth profiles of O_2 concentration in dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars are standard errors (n = 12)

centrations during the dry season were; 1.09 (0.04) ppm at 10 cm, 1.09 (0.07) ppm at 30 cm and 0.98 (0.02) ppm at 50 cm in the ultisols and 1.32 (0.08) ppm at 10 cm, 1.19 (0.09) ppm at 30 cm and 1.14 (0.05) ppm at 50 cm in the inceptisols. Similarly during the wet season, mean concentrations were: 1.29 (0.07) ppm at 10 cm, 1.18 (0.05) ppm at 30 cm and 1.29 (0.13) ppm at 50 cm in the ultisols and 1.76 (0.16) ppm at 10 cm, 1.81 (0.17) ppm at 30 cm and 1.85 (0.25) ppm at 50 cm in the inceptisols.

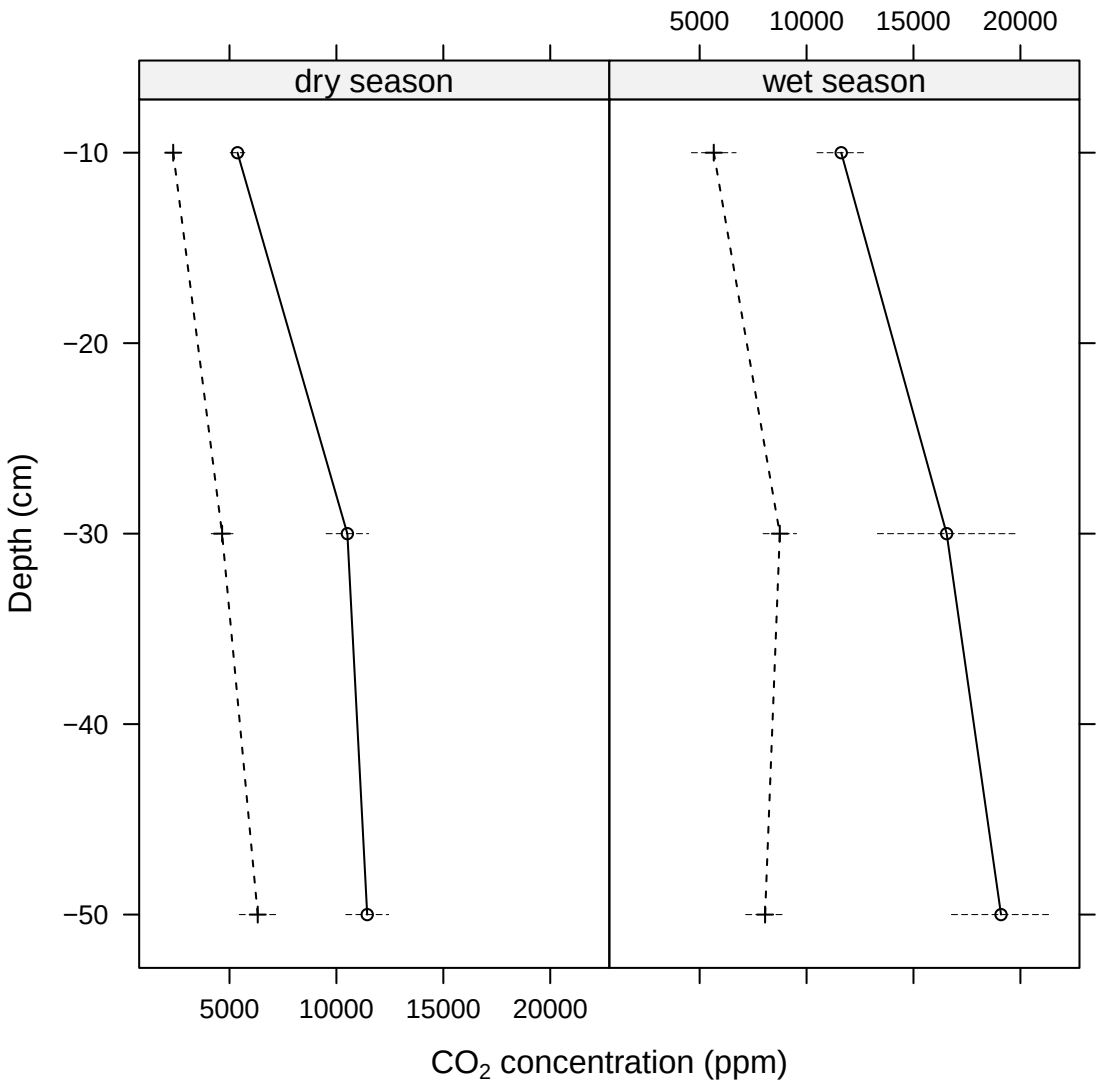

Figure 2.5: Depth profiles of CO_2 concentration in dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars standard errors (n = 12).

2.4.2.3 CH_4 and CO_2 flux rates

There was no clear difference in CO_2 flux rate between sites (Table 2.3). CO_2 flux rates were greater in the wet season than the dry season with, respective, mean rates of 4.28 (0.15) and 2.90 (0.09) g C-CO_2 m^{-2} d^{-1} from the inceptisols and 5.15 (0.15) and 2.50 (0.08) g C-CO_2 m^{-2} d^{-1} from the ultisols. During both seasons, CO_2 flux rate varied considerably between days and within sites (Figure 2.7).

The soils at both sites principally acted as a sink for atmospheric CH_4 (Table 2.3). Uptake of atmospheric CH_4 was greater, with lower mean flux rates, in the ultisol soils than in the inceptisol soils. Flux rate was greater in the wet than the dry season with

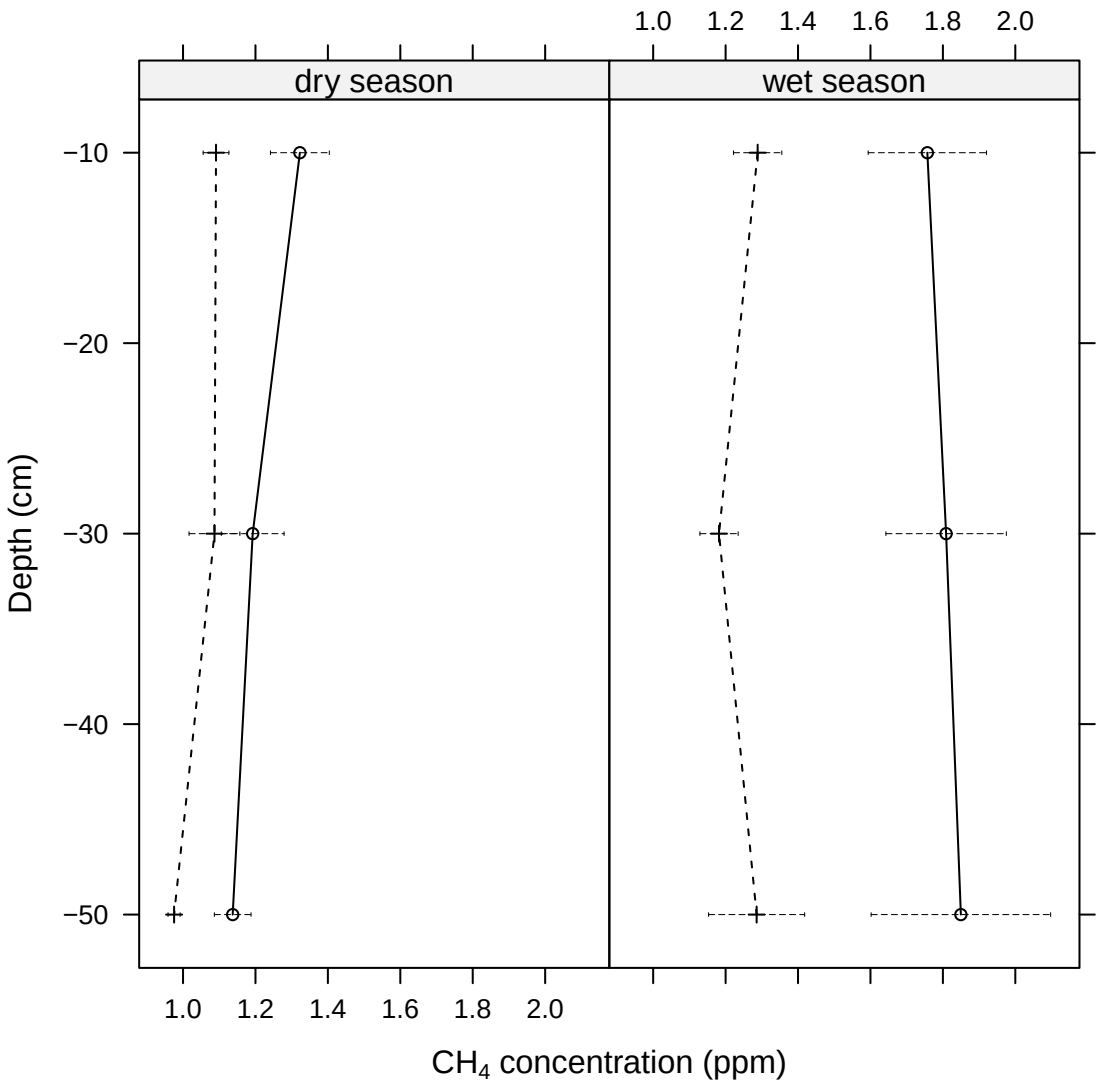

Figure 2.6: Depth profiles of CH_4 concentration in dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars are standard errors (n = 12).

means of -0.41 (0.10) and -0.95 (0.06) mg C-CH_4 m^{-2} d^{-1} for the inceptisols and - 1.39 (0.07) and -1.56 (0.06) mg C-CH_4 m^{-2} d^{-1} for the ultisols, respectively. There was little seasonal variation in the distribution of fluxes observations between emission, uptake and no flux in the ultisol soils with uptake accounting for 77 and 72 % of measurements in dry and wet season, respectively (Table 2.4). In contrast, observations of emission and no flux are both, respectively, 13 % higher in wet season than dry in the inceptisol soils. In both wet and dry season day to day variations in CH_4 flux rate were similar between sites (Figure 2.8). Temporal and spatial variations in CH_4 flux rate were greater during the wet than dry season. During the wet season, within site variability in daily mean flux rate was typically greater than day to day variations.

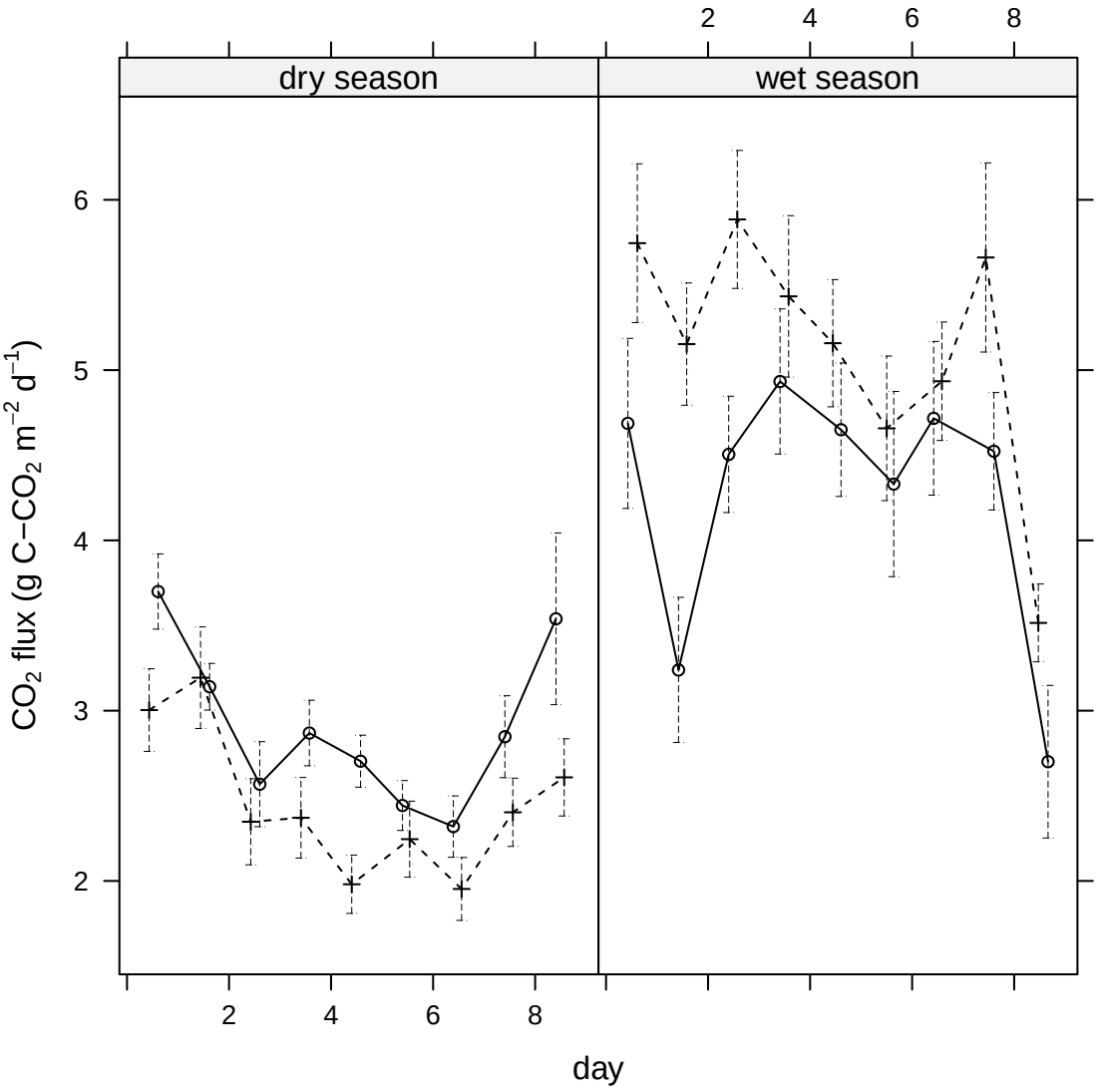

Figure 2.7: Daily averaged CO_2 flux rate for both sites during dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars are standard errors (n = 12).

During the dry season, within site variability was high but day to day variations were more similar.

2.4.3 Relationships between CH_4 cycling and other environmental variables

Among sites and season, CH_4 flux rate was significantly positively correlated with WFPS (Spearman's ρ = 0.46, p < 0.05) and negatively correlated with soil O_2 concentration at 10 cm (Spearman's ρ = -0.18, p < 0.05), 30 cm (Spearman's ρ = -0.26, p < 0.05) and 50 cm (Spearman's ρ = -0.22, p < 0.05). No significance was found in

Table 2.4: Classification of CH_4 fluxes for each campaign, grouped by site, in dry and wet season. Reported mean fluxes and environmental relationships are calculated from emission and uptake categories. Reported proportion of fluxes where no flux was measured are calculated from the no flux category. Fluxes in the NA category are excluded from analysis. Values are % (number).

	Site	Emission	Uptake	No flux	NA	Total
dry	Inceptisol	0.00 (0)	58.33 (63)	34.26 (37)	7.41 (8)	100.00 (108)
season	Ultisol	0.00 (0)	76.85 (83)	20.37 (22)	2.73 (3)	100.00 (108)
wet	Inceptisol	12.96 (14)	36.11 (39)	47.22 (51)	3.70 (4)	100.00 (108)
season	Ultisol	0.93 (1)	72.22 (78)	18.52 (20)	8.33 (9)	100.00 (108)

weaker correlations between CH_4 flux and CO_2 flux (Spearman's ρ = 0.01, p = 0.93), air temperature (Spearman's ρ = 0.09, p = 0.16) or soil temperature (Spearman's ρ = 0.10, p = 0.09). These Spearman's rank correlation coefficients are reported in Table 2.5. The minimal valid linear model explaining CH_4 flux is a positive correlation with WFPS and negative correlation with CO_2 flux (r^2 = 0.30, p < 0.05). In this context, WFPS accounted for the majority of the explained variance (Figure 2.9, r^2 = 0.26, p < 0.05).

Between seasons in the inceptisol soils, CH_4 flux was significantly positively correlated with WFPS (Spearman's ρ = 0.47, p < 0.05), air temperature (Spearman's ρ = 0.31, p < 0.05), soil temperature at 5 cm (Spearman's ρ = 0.28, p < 0.05) and CO_2 flux rate (Spearman's ρ = 0.02, p < 0.05) and negatively correlated with soil O_2 concentration at 10 cm (Spearman's ρ = -0.34, p < 0.05), 30 cm (Spearman's ρ = -0.39, p < 0.05) and 50 cm (Spearman's ρ = - 0.41, p < 0.05). These Spearman's rank correlation coefficients are reported in Table 2.6. Similarly to the complete dataset, stepwise linear regression indicates the minimal model explaining CH_4 flux is a positive correlation with WFPS and a negative correlation with CO_2 flux rate (r^2 = 0.25, p < 0.05). The positive correlation with WFPS accounted for the majority of the explained variance (r^2 = 0.22, p < 0.05). The strongest relationship between soil gas concentrations is at 30 cm depth. The minimal valid linear model explaining CH_4 concentration is the negative correlations with both O_2 and CO_2 concentration (r^2 = 0.50, p < 0.05).

Between seasons in the ultisol soils, CH_4 flux rate was not significantly correlated with WFPS (Spearman's ρ = 0.09, p = 0.28), air temperature (Spearman's ρ = 0.13, p = 0.11), soil temperature at 5 cm (Spearman's ρ = 0.07, p = 0.39), CO_2 flux rate (Spear-

Table 2.5: Spearman's rank correlation coefficients for correlations among soil type and season (n = 278). Values are ρ where negative values indicate inverse correlations and significance at $p < 0.05$ is signified by *.

All data	CH_4 flux	CO_2 flux	WFPS	O_2 10 cm	O_2 30 cm	O_2 50 cm	T air	T soil
CH_4 flux	NA	0.01	0.46*	-0.18*	-0.26*	-0.22*	0.09	0.10
CO_2 flux	0.01	NA	0.41*	-0.62*	-0.57*	-0.60*	0.65*	0.72*
WFPS	0.46*	0.41*	NA	-0.55*	-0.67*	-0.62*	0.34*	0.47*
O_2 10 cm	-0.18*	-0.62*	-0.55*	NA	0.84*	0.85*	-0.68*	-0.78*
O_2 30 cm	-0.26*	-0.57*	-0.67*	0.84*	NA	0.85*	-0.67*	-0.77*
O_2 50 cm	-0.22*	-0.60*	-0.62*	0.85*	0.85*	NA	-0.67*	-0.81*
T air	0.09	0.65*	0.34*	-0.68*	-0.67*	-0.67*	NA	0.85*
T soil	0.10	0.72*	0.47*	-0.78*	-0.77*	-0.81*	0.85*	NA

Table 2.6: Spearman's rank correlation coefficients for correlations between season for inceptisol soils (n = 116). Values are ρ where negative values indicate inverse correlations and significance at $p < 0.05$ is signified by *.

inceptisols	CH_4 flux	CO_2 flux	WFPS	O_2 10 cm	O_2 30 cm	O_2 50 cm	T air	T soil
CH_4 flux	NA	0.02	0.47*	-0.34*	-0.39*	-0.41*	0.31*	0.28*
CO_2 flux	0.02	NA	0.33*	-0.46*	-0.51*	-0.47*	0.63*	0.64*
WFPS	0.47*	0.33*	NA	-0.62*	-0.84*	-0.72*	0.64*	0.65*
O_2 10 cm	-0.34*	-0.46*	-0.62*	NA	0.78*	0.80*	-0.67*	-0.72*
O_2 30 cm	-0.39*	-0.51*	-0.84*	0.78*	NA	0.79*	-0.74*	-0.77*
O_2 50 cm	-0.41*	-0.47*	-0.72*	0.80*	0.79*	NA	-0.76*	-0.80*
T air	0.31*	0.63*	0.64*	-0.67*	-0.74*	-0.76*	NA	0.87*
T soil	0.28*	0.64*	0.65*	-0.72*	-0.77*	-0.80*	0.87*	NA

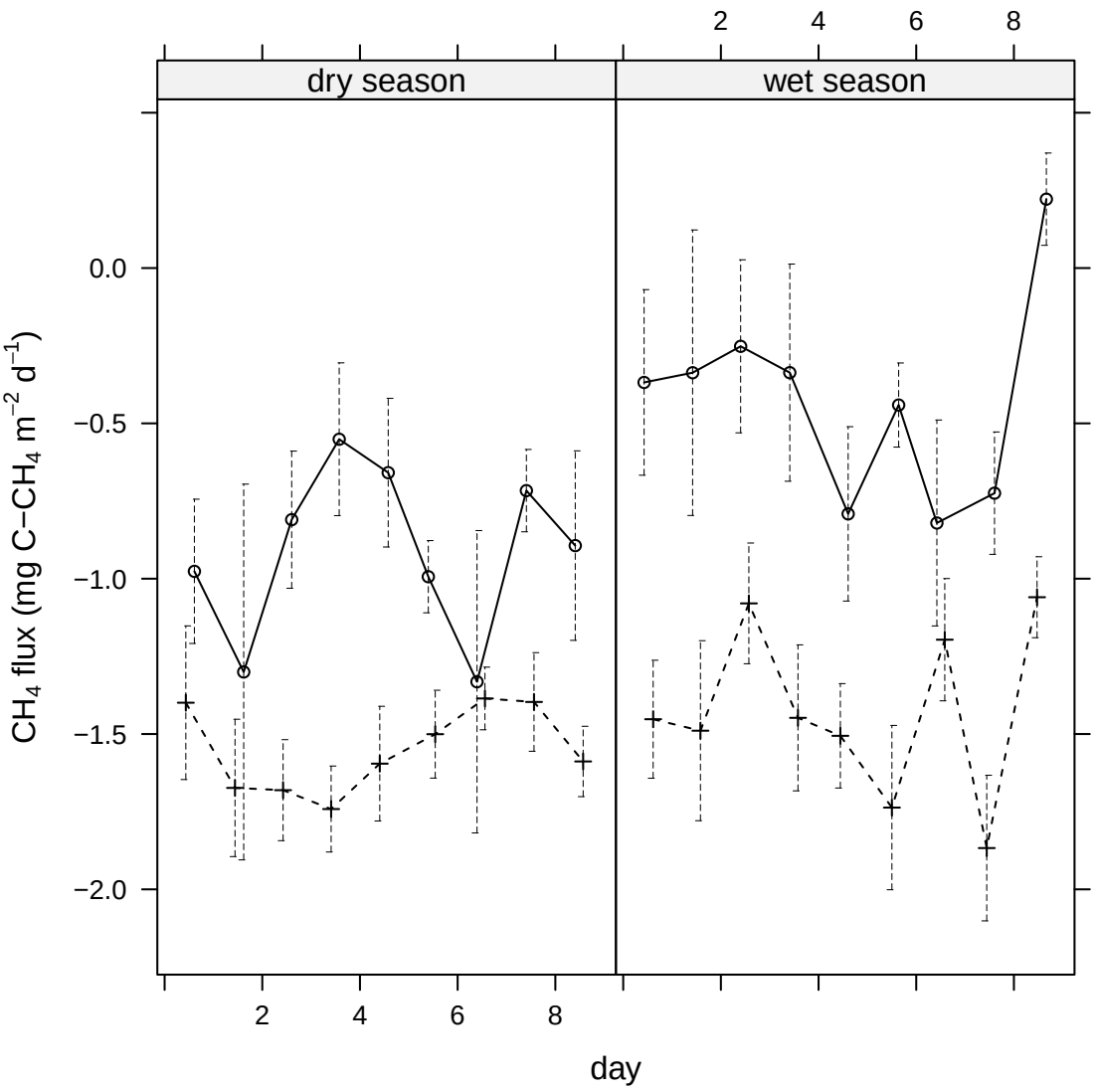

Figure 2.8: Daily averaged CH_4 flux rate for both sites during dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars standard errors (n = 12).

man's ρ = -0.03, p = 0.70) or soil O_2 concentration at 10 cm (Spearman's ρ = -0.14, p = 0.07), 30 cm (Spearman's ρ = -0.07, p = 0.36) or 50 cm (Spearman's ρ = -0.05, p = 0.51). These Spearman's rank correlation coefficients are reported in Table 2.7. Similarly, no valid statistically significant linear relations were found between CH_4 flux and environmental variables. The strongest relationship between soil gas concentrations is at 10 cm depth. The minimal valid model explaining CH_4 concentration is the negative correlations with both O_2 and CO_2 concentration and a positive interaction between O_2 and CO_2 concentration (r^2 = 0.45, p < 0.05).

Table 2.7: Spearmans rank correlation coefficients for correlations between season for ultisol soils (n = 162). Values are ρ where negative values indicate inverse correlations and significance at $p < 0.05$ is signified by *.

ultisols	CH_4 flux	CO_2 flux	WFPS	O_2 10 cm	O_2 30 cm	O_2 50 cm	T air	T soil
CH_4 flux	NA	-0.03	0.09	-0.14	-0.07	-0.05	0.13	0.07
CO_2 flux	-0.03	NA	0.53*	-0.69*	-0.63*	-0.67*	0.68*	0.78*
WFPS	0.09	0.53*	NA	-0.64*	-0.61*	-0.64*	0.44*	0.55*
O_2 10 cm	-0.14	-0.69*	-0.64*	NA	0.89*	0.87*	-0.71*	-0.81*
O_2 30 cm	-0.07	-0.63*	-0.61*	0.89*	NA	0.88*	-0.70*	-0.80*
O_2 50 cm	-0.05	-0.67*	-0.64*	0.87*	0.88*	NA	-0.66*	-0.83*
T air	0.13	0.68*	0.44*	-0.71*	-0.70*	-0.66*	NA	0.83*
T soil	0.07	0.78*	0.55*	-0.81*	-0.80*	-0.83*	0.83*	NA

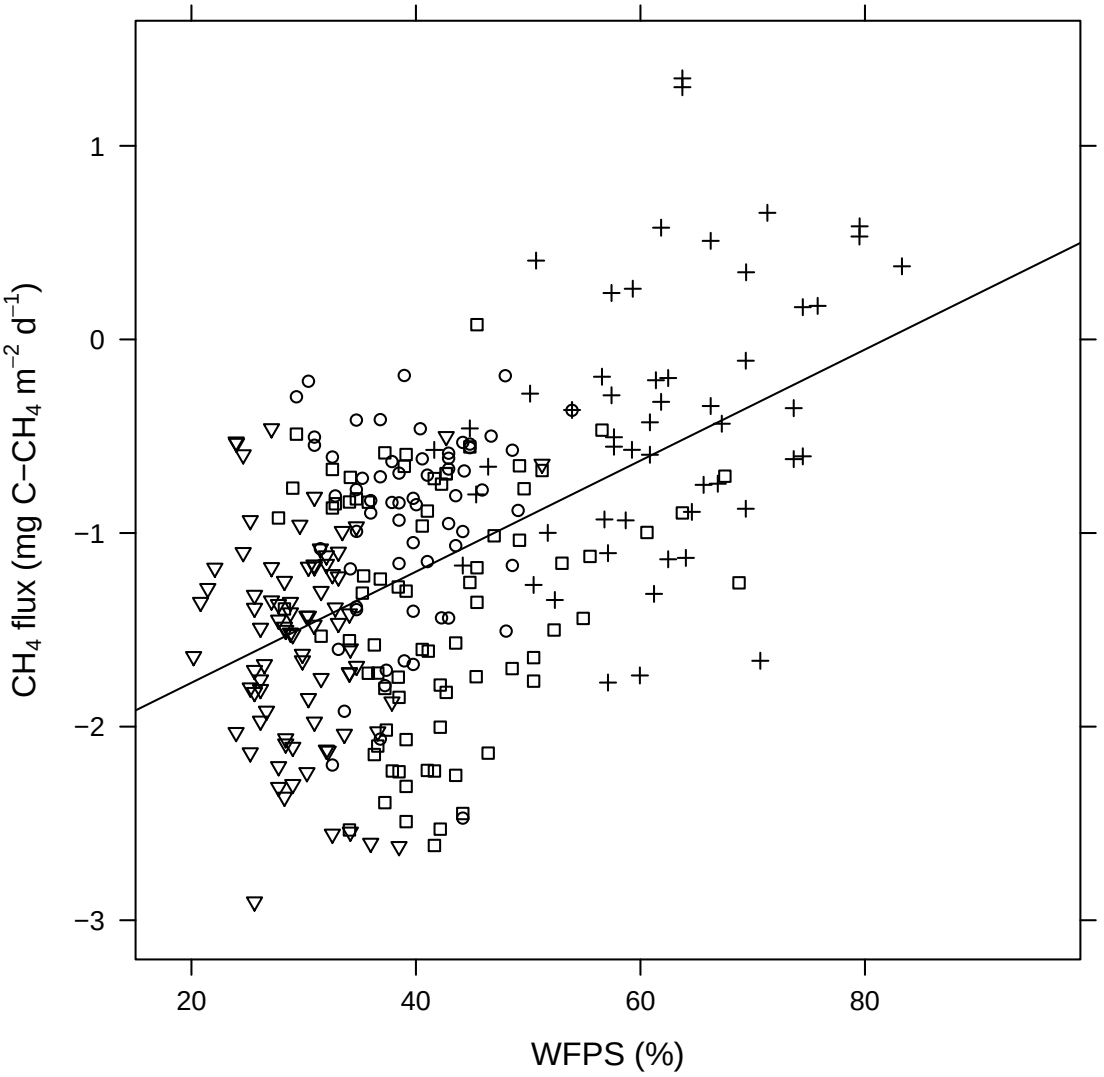

Figure 2.9: Net CH_4 flux vs WFPS for all data among soil type and season (n = 278). Points: ultisols; dry season = $\triangledown$ and wet season = □, inceptisols; dry season = ∘ and wet season= +. Linear regression line: net CH_4 flux = 0.03 * WFPS - 2.35 , r^2 = 0.26.

2.4.4 Gross process rates

In vitro net and partitioned gross CH_4 flux rates by site and depth are summarised in Table 2.8. Rates of consumption, *C*, were typically greater than rates of production, *P*, leading to a decline in headspace CH_4 concentration and negative net fluxes, *F*, between -0.02 and -0.33 nmol g dry soil^{-1} d^{-1}. With depth, consumption rates appear more variable than production rates with large changes in net flux associated with differences in consumption rate. Within the inceptisol soils, gross process rates were most dissimilar at 0 - 5 cm with a net flux rate of -0.34 nmol g dry soil^{-1} d^{-1}, corresponding

to consumption and production rates of 0.73 and 0.39 nmol g dry soil^{-1} d^{-1}, respectively. Gross process rates were most similar at 25 - 35 cm with a net flux rate of -0.07 nmol g dry soil^{-1} d^{-1} corresponding to consumption and production rates of 0.34 and 0.27 nmol g dry soil^{-1} d^{-1}, respectively. Within the ultisol soils, gross process rates were most dissimilar at 5 - 15 cm with a net flux rate of -0.26 nmol g dry soil^{-1} d^{-1} corresponding to consumption and production rates of 0.58 and 0.33 nmol g dry soil^{-1} d^{-1}, respectively. Gross process rates were most similar at 0 – 5 cm with a net flux rate of -0.02 nmol g dry soil^{-1} d^{-1} corresponding to consumption and production rates of 0.34 and 0.32 nmol g dry soil^{-1} d^{-1}, respectively. No significant relationships were identified between physical, chemical or environmental soil properties and gross process rates. The model represents a reasonable fit to the data with r^2 of 0.99 and 0.92 for linear regressions of modelled predictions against observed changes in CH_4 headspace concentration and the atom $\%$ of ^{13}C - CH_4, respectively (Figure 2.10).

Table 2.8: Net and gross CH_4 process rates by site and depth. Values are mean (standard error). n = 6 per site and depth.

Site	Depth (cm)	Net flux (nmol CH_4 g^{-1} d^{-1})	Consumption (nmol CH_4 g^{-1} d^{-1})	Production (nmol CH_4 g^{-1} d^{-1})
Inceptisol soils	0 - 5	-0.34 (0.10)	0.73 (0.11)	0.39 (0.01)
	5 - 15	-0.17 (0.09)	0.40 (0.09)	0.23 (0.03)
	25 - 35	-0.07 (0.03)	0.34 (0.01)	0.27 (0.03)
	45 - 55	-0.11 (0.01)	0.43 (0.07)	0.32 (0.07)
Ultisol soils	0 - 5	-0.02 (0.01)	0.34 (0.06)	0.32 (0.07)
	5 - 15	-0.25 (0.08)	0.58 (0.04)	0.33 (0.05)
	25 - 35	-0.05 (0.06)	0.35 (0.08)	0.30 (0.06)
	45 - 55	-0.04 (0.01)	0.38 (0.12)	0.34 (0.10)

2.5 Discussion

2.5.1 Spatial and temporal variations in net CH_4 flux

Soil-atmosphere CH_4 exchange among site and season showed a similar pattern, with greater net fluxes from soils with lower porosity and higher WFPS, to that observed for upland forests elsewhere in the Amazon basin. In this study, wet and dry season mean net fluxes were, respectively, -0.41 (0.10) and -0.95 (0.06) mg C-CH_4 m^{-2} d^{-1}

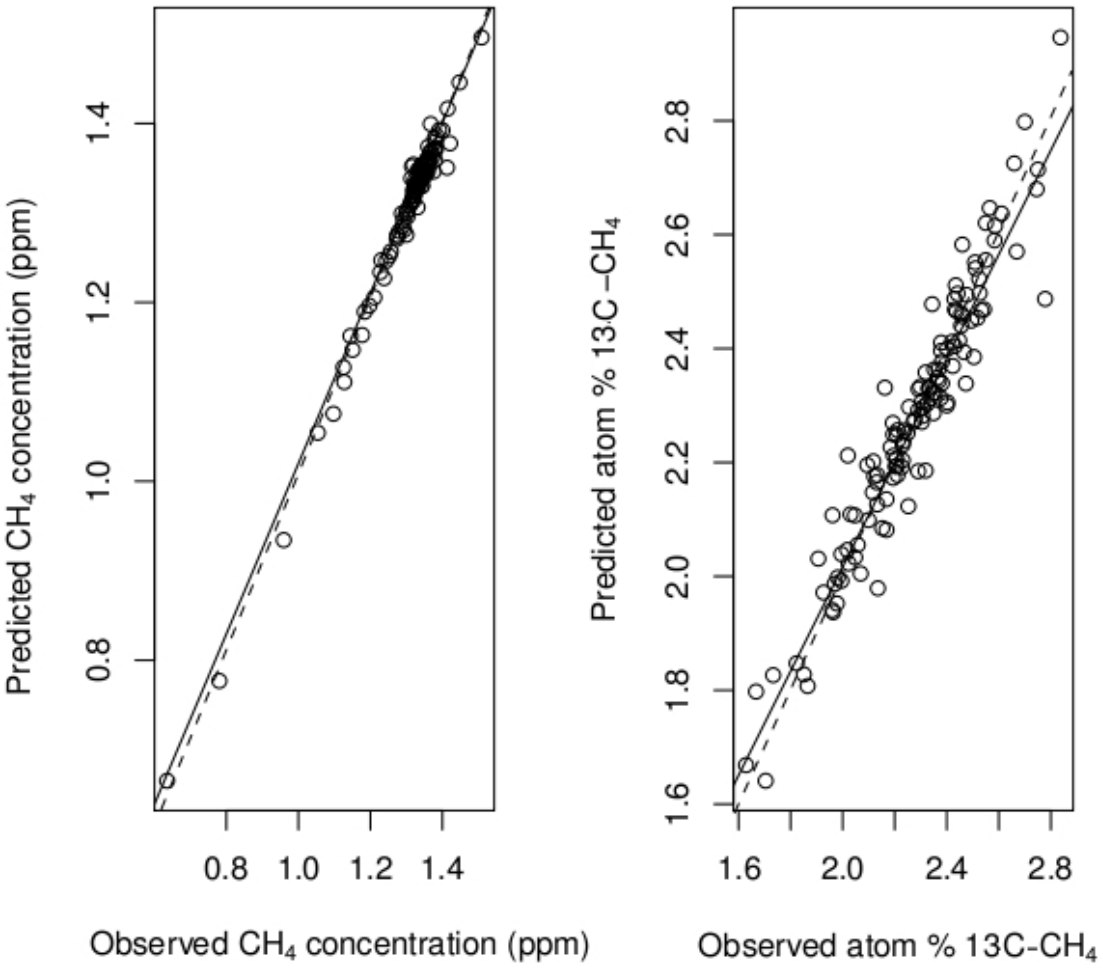

Figure 2.10: Pooled comparison of predicted against observed CH_4 concentration and atom %. Fitted linear relationships and 1:1 lines and between predicted and observed values are indicated by solid and dashed lines, respectively.

for the lower porosity inceptisol soils and -1.39 (0.07) and -1.56 (0.06) mg C-CH_4 m^{-2} d^{-1} for the higher porosity ultisol soils. In comparison, mean annual fluxes ranging from -0.20 to -1.22 mg C-CH_4 m^{-2} d^{-1} for coarse textured ultisol soils (Steudler et al., 1996; Fernandes et al., 2002; Keller et al., 2005) and -0.06 to -0.49 mg C-CH_4 m^{-2} d^{-1} (Keller et al., 1986; Verchot et al., 2000; Keller et al., 2005; Davidson et al., 2008) for fine textured oxisols have been previously reported for forested uplands in the Brazilian Amazon.

The response to season differs between soil type with net flux from the inceptisol soils almost doubling between dry and wet season campaigns, whilst, the change observed for the ultisol soils was more marginal. The increase in net flux in the inceptisol soils appears to be linked to both a decrease in uptake rates and a shift in the balance between

methanogenesis and methanotrophy in favour of production during the wet season with the proportion of flux measurements that were emission increasing by ~ 13 %. Such a shift is also supported by the increase in soil CH_4 concentrations from below to close to atmospheric levels between dry and wet season. In this respect, emission events are presumably driven by the observation that soil CH_4 concentration exceeded atmospheric levels at some sampling locations. In contrast, the slight increase in net flux between dry and wet season in the ultisol soils appears to be driven by methanotrophic activity with no apparent increase in emissions and only a slight elevation in soil CH_4 concentration. These findings are consistent with previous studies indicating wet season decreases in methanotrophy and increased methanogenesis at some sites (Keller et al., 1986; Verchot et al., 2000; Davidson et al., 2008). In this context, for example, Keller et al. (2005) report mean wet and dry season fluxes of 0.06 (0.04) and - 0.20 (0.06) mg C-CH_4 m^{-2} d^{-1} from a fine textured oxisol and -0.14 (0.06) and -0.29 (0.08) mg C-CH_4 m^{-2} d^{-1} from a coarse textured ultisol. Within season, spatial variations in net CH_4 were typically greater than that between daily measurements indicating factors unique to sampling locations are likely more important than variations in environmental conditions at day to week timescales. In these respects, the soils of our study sites in the Peruvian, Western Amazon appear, qualitatively, to function similarly to other upland tropical forests in the Amazon basin.

2.5.1.1 Uncertainties in net CH_4 flux

Assessing soil-atmosphere CH_4 exchange based on short-term measurement campaigns is difficult as environmental conditions may vary substantially on an intra and inter-annual basis. Whilst no published long-term environmental data is yet available for the inceptisol soils, the ultisol soils are proximal to the long-term RAINFOR site TAM 05 which is the subject of intensive research into forest dynamics and soil C cycling (Zimmermann et al., 2010a; Girardin et al., 2010; Malhi et al., 2013). Of particular relevance in bracketing the findings of this study in larger scale variations the environment may experience is work by Malhi et al. (2013) which documented monthly variations, between 2005 and 2011, in CO_2 flux, volumetric water content and air temperature

as part of a holistic study of autotrophic and heterotrophic C cycling at TAM 05 and another long term site in the area. We report similar patterns to Malhi et al. (2013) with higher CO_2 fluxes, WFPS and air temperature in the wet than dry season. Wet and dry season mean CO_2 fluxes from the ultisol soils in this study were, respectively, 5.15 (0.15) and 2.50 (0.08), whilst, an annual mean of 3.32 (0.02) g C-CO_2 m^{-2} d^{-1} with a wet season maximum of ~ 6 g C-CO_2 m^{-2} d^{-1} and dry season minimum of ~ 1 g C-CO_2 m^{-2} d^{-1} is reported for TAM 05 between 2005 and 2011 (Malhi et al., 2013). We observed an increase in mean WFPS from 30.6 (0.5) to 43.2 (0.9) $\%$ between dry and wet season, whilst, long term measurements at TAM 05 indicate equivalent, when converted from volumetric water content to WFPS using porosity data reported in this study, seasonal values of ~ 30 and 50 $\%$. Mean air temperature during the wet and dry season in this study was, respectively, 28.2 (0.2) and 22.8 (0.1) $^{\circ}C$, whilst, Malhi et al. (2013) report a long term mean of 24.4 $^{\circ}C$ with a wet season maximum of 26.5 $^{\circ}C$ and dry season minimum of 21.5 $^{\circ}C$. In this context, the reported CH_4 fluxes appear to be broadly representative of the seasonal differences in activity for this environment, however, the data does not represent end-member conditions and, as such, do not preclude more extreme variations, such as, greater CH_4 emissions later in the wet season or increased uptake in drought years (Davidson et al., 2008).

Similarly to other studies, daily time-series of CH_4 flux indicate considerable spatial variability within site and season (Verchot et al., 2000; Davidson et al., 2008). In this context, spatial variations within days were greater than variations between days at the measured time-scales (Figure 2.8). This is particularly true for the wet season campaigns where both spatial and temporal variations were more pronounced than in the dry season (Keller et al., 2005). These differences may result from a number of possibilities. Firstly, measurements were taken within the same plots and sites in each measurement campaign but the sampling locations, although proximal, were not the same. This approach could have resulted in the introduction of greater spatial variability during the wet season in the form of hotspots of microbial activity, with Verchot et al. (2000) estimating that mean CH_4 flux determined from 8 locations had a 95 $\%$ chance of being within 300 $\%$ of a mean determined from 36 sampling locations in an Brazilian upland tropical forest. Secondly, the proportion of flux rates that were un-

quantifiable, termed 'no flux', and subsequently removed from the dataset varied at the level of sampling location and day. No flux observations accounted for 20 and 34 % of the flux measurements in the dry season and 19 and 47 % of observations in the wet season from the ultisol and inceptisol soils, respectively (Table 2.4). Removal of these values suggests that the true seasonal means are closer to zero than the reported values and that assessing temporal variability within soil type and season is difficult as time series for each sampling location, and thus daily site means, are unbalanced and centrally censored with 'missing not at random' observations (Wu, 2009). In this context, whilst the increase in weak, unquantifiable emission or uptake fluxes is in keeping with the observed shift to a more positive mean flux rate in the wet season for the inceptisol soils there is also less confidence in the reported mean (Verchot et al., 2000). Finally, as a similar pattern is seen between dry and wet season for both soil types, despite there being no large change in no flux observations for the ultisol soils, the increase in wet season variability may reflect spatial differences in the biophysical constraints on CH_4 flux in response to tandem increases in variability observed in environmental parameters such as WFPS.

2.5.2 Contrasts in drivers of net CH_4 flux

Higher net CH_4 flux rates from finer textured soils and wet season shifts to lower uptake rates, potentially leading to transient periods of emission, from tropical upland soils have been explained by the influence of diffusivity on CH_4 and O_2 availability. In this model, constraints on diffusivity imposed by a combination of soil pore structure and WFPS regulate consumption through the supply of CH_4 to methanotrophic bacteria and production through the extent of anaerobic microsites suitable for methanogenic microbial communities in response to ingress and uptake by aerobic respiration of O_2 (Verchot et al., 2000; Smith et al., 2003). In this context, we hypothesized that soil O_2 concentration, as an integrated proxy for diffusion and biological O_2 demand, would best predict net CH_4 flux. Contrary to this, we reject our hypothesis, for the sites and time-scales encompassed by this study, as the strongest correlation, among soil type and season, between measured parameters and CH_4 flux was with WFPS (Spear-

man's ρ = 0.46). The minimal valid linear model explaining the data was a positive relationship with WFPS and a negative relationship with CO_2 flux (r^2 = 0.30) with the majority of the explained variance accounted for by WFPS (r^2 = 0.26). Such a relationship supports previous conclusions that WFPS as a function of porosity is important in determining differences in net CH_4 between soil types (Verchot et al., 2000; Smith et al., 2003). Within sites, the same pattern emerges for the inceptisol soils, whilst, no statistically significant relationships between CH_4 and measured environmental parameters was found across season for the ultisol soils. This discrepancy supports the observed differences in fluxes among soil type and season in this study and suggests that there are both between and within soil type differences in the response of the processes governing net CH_4 exchange to seasonal changes in WFPS (Hall et al., 2013).

For the ultisol soils, a slight increase in net CH_4 flux and no increase in emissions or soil CH_4 concentrations above the atmospheric background between dry and wet season was observed indicating that methanogenesis may not play an important role in determining net soil-atmosphere exchange at this site. No statistically significant relationships between net CH_4 flux and environmental conditions were found between seasons in these soils suggesting the response of methanotrophy to the seasonal increase in WFPS within this site is complex. Methanotrophs involved in the uptake of CH_4 from the atmosphere are expected to occupy ecological niches within well-connected macro-pores to facilitate their dependence on both oxic conditions and diffusive supply of CH_4 from the atmosphere (Von Fischer et al., 2009). Methanotrophic processes are micro-aerophilic, capable of operating at O_2 concentrations > 3 $\%$ and as we find no evidence for significant anoxia in the bulk soil matrix this suggests O_2 availability is unlikely to influence uptake rates within these soils (Teh et al., 2006). We would typically expect to associate increases in WFPS with decreases in uptake as diffusional constraints act to limit the supply of CH_4 from the atmosphere, despite this, no such relationship was identified. Similarly, whilst paired, measurements of net CH_4 flux and soil CH_4 concentration were not made we may expect diffusional limitations to be generally reflected in our measurements of O_2 concentration. Indeed, contrary to this there is in fact an increase in both WFPS and soil CH_4 concentration between dry and wet season in these soils. Our inability to identify relationships between net CH_4 flux

and environmental parameters may reflect spatial contrasts in the relationship between WFPS and the biophysical constraints on methanotrophy, with for example diffusional limitations increasing net flux at some sampling locations whilst at other locations alleviation of water stress may act to promote methanotrophy and decrease net fluxes (Von Fischer et al., 2009).

In the inceptisol soils, increased net CH_4 flux, emission observations and soil CH_4 concentrations from significantly below to close to atmospheric background levels, suggests that difference between dry and wet season is driven by constraints on methanotrophic activity involved in uptake of atmosphereic CH_4 and a shift in the balance between methanotrophy and methanogenesis in favour of production. The minimal valid linear model explaining net CH_4 flux is a positive correlation with WFPS and a negative correlation with CO_2 flux rate (r^2 = 0.25), where WFPS alone explains 22 $\%$ of the variance. The microbial communities involved in consumption and production are expected to exploit contrasting ecological niches, with methanotrophs occupying well connected pore-spaces whilst methanogens populate spatially segregated anoxic microsites. In this context and as, similarly to the ultisol soils, we do not observe significant depletion of O_2 in the bulk soil matrix likely to suppress methanotrophic activity, this relationship may be linked to the supply of CH_4 to methanotrophs consuming atmospheric CH_4 and the promotion of methanogenesis (Conrad, 1996; Teh et al., 2005; Von Fischer and Hedin, 2007). Such relationships with WFPS and CO_2 are traditionally explained as relating to constraints on the inward diffusion from the atmosphere and expansion of anaerobic microsites leading to the suppression of aerobic respiration and thus decreases in CO_2 flux (Schuur et al., 2001; Verchot et al., 2000; Cleveland et al., 2010). Whilst WFPS appears to be the principal driver of variations in net CH_4 flux in this site (Spearman's ρ = 0.46), weaker negative co-correlations with soil O_2 concentration (Spearman's ρ = - 0.34 at 10 cm, - 0.39 at 30 cm and - 0.41 at 50 cm) within the soil profile may support such a mechanism for the promotion of methanogenesis. The fact that, contrary to our hypothesis, WFPS better reflects variations in net CH_4 flux than O_2 concentrations suggests that variations in the activity of methanotrophs consuming atmospheric CH_4 are principally determining the activity of these soils and that O_2 availability in the intrinsically well-connected pore spaces

of the bulk soil matrix poorly characterises methanogenic activity within micro-sites. This may indicate that spatial heterogeneity in resource availability, either linked to areas of abundant labile C related to aggregates and the rhizosphere (Wachinger et al., 2000; Whiting and Chanton, 1993) or through competition with more energetically favourable metabolisms (Von Fischer and Hedin, 2007; Teh et al., 2008a) is more important than anaerobic volume in determining the response of methanogenic activity, and thus net CH_4 flux, to more favourable conditions in the wet season (Sexstone et al., 1985; Hall et al., 2013).

2.5.3 Below-ground CH_4 cycling

Differences, among soil type and season, in soil-atmosphere CH_4 exchange and its relationship to environmental parameters suggests that net CH_4 flux may be driven by methanotrophy in the ultisol soils and and both methanotrophy and methanogenesis in the inceptisol soils during the study period. In this context, it appears that spatial variations in the biophysical controls on consumption and production in the tropical upland forest soils studied are complex (Teh et al., 2005; Von Fischer and Hedin, 2007; Hall et al., 2013). The isotope pool dilution incubations indicate that production does occur in these soils despite oxic conditions supporting the field observations of this study and previous evidence of the existence of anaerobic microsites in similar environments (Andersen et al., 1998; Verchot et al., 2000; Von Fischer and Hedin, 2002). In the ultisol soils, the minimal valid model explaining soil CH_4 concentrations, which were below atmospheric concentrations in both seasons, is negative correlations with both O_2 and CO_2 concentration and a positive interaction between O_2 and CO_2 concentration (r^2 = 0.45) at 10 cm. Gross process rates were most dissimilar and consumption at its maximum at 5 - 15 cm in these soils with a net flux rate of -0.26 nmol g dry soil^{-1} d^{-1} corresponding to consumption and production rates of 0.58 and 0.33 nmol g dry soil^{-1} d^{-1} , respectively. This suggests that variations in net CH_4 in the ultisols is linked to the activity of methanotrophs in the mineral soils directly below the thin organic horizon found in these environments (Zimmermann et al., 2009). Similarly in the inceptisol soils, gross process rates were most dissimilar and consumption was greatest

in surface soils from 0 - 5 cm depth with a net flux rate of -0.33 nmol g dry soil^{-1} d^{-1} , corresponding to consumption and production rates of 0.73 and 0.39 nmol g dry soil^{-1} d^{-1}, respectively. However, in these soils the minimal valid model explaining soil CH_4 concentration, which increased from below to close to atmospheric background in the upper 30 cm between dry and wet season, was a negative correlation with O_2 and CO_2 at 30 cm depth (r^2 = 0.50). Gross process rates were also most similar in soils from 25 - 35 cm depth at this site with a net flux rate of -0.07 nmol g dry soil^{-1} d^{-1} corresponding to consumption and production rates of 0.34 and 0.27 nmol g dry soil^{-1} d^{-1}, respectively. This suggests the inference that the seasonal shift observed for these soils resulting from diffusional constraints on the supply of CH_4 and the expansion of anaerobic microsites may respectively reflect differences in activity at the surface and at depth. In these contexts, the differences in observations between soil types may reflect threshold effects with diffusion not becoming sufficiently limited in the higher porosity ultisol soils to promote significant changes at depth.

2.6 Conclusion

We find that the studied soils principally acted as a sink for atmospheric CH_4 during the measurement period. Differences between soil types followed previously observed patterns with greater uptake in the higher porosity ultisol than lower porosity inceptisol soils. Incubations indicate that methanogenesis appears to be occurring in both soil types despite oxic conditions in the bulk soil matrix. However, we only find evidence of a seasonal shift in the supply of CH_4 to methanotrophs consuming atmospheric CH_4 and the balance of consumption and production, leading to emissions, in the inceptisol soils. This shift is best explained by changes in WFPS rather than O_2, whilst no significant relationships are found between net CH_4 flux and environmental parameters in the ultisol soils suggesting the controls on both methanotrophy and methanogenesis in these environments is complex. Considering the potential for deviations from the expected patterns of CH_4 uptake in such environments this indicates that understanding the factors governing methanogenic activity is important in understanding spatial and temporal variability in fluxes from such soils.

Chapter 3

Uptake of atmospheric methane by premontane and montane forest soils in the southern Peruvian Andes

Sam Jones[1], Torsten Diem[3], Lidia Huaraca Quispe[2],Patrick Meir[1] and Yit Arn Teh[3]

[1]School of Geosciences, University of Edinburgh, Edinburgh, United Kingdom, [2]Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru, [3]Institute of Biological and Environmental Sciences, University of Aberdeen, United Kingdom

This chapter reports soil-atmosphere CH_4 exchange and soil environmental conditions for premontane and montane forests in the Peruvian Andes. This data was collected as part of a NERC grant entitled 'Are tropical uplands regional hotspots for methane and nitrous oxide?'. Here we report the full period of measurements, January 2010 to June 2013, for this dataset. Supplementary data can be found in Appendix C. The first year of this data has previously been published as Teh et al. (2014). A copy of this publication can be found in Appendix E. Contributions to this chapter have been made by Sam Jones, Torsten Diem, Lidia Huaraca Quispe, Patrick Meir and Yit Arn Teh. Yit Arn Teh and Patrick Meir won the funding for this work and outlined the basis of the experimental design. Torsten Diem orchestrated the installation of field sites and data collection with the assistence of Lidia Huaraca Quispe and Sam Jones. Sam Jones designed and constructed the soil-gas equilibration chambers and developed the protocol for field measurements of soil oxygen concentration. Lidia

Huaraca Quispe was responsible for monthly data collection. Gas chromatography analyses and calculation of net flux rates was conducted by Torsten Diem. Statistical analysis and writing was conducted by Sam Jones.

3.1 Abstract

Soil-atmosphere CH_4 exchange in tropical landscapes is poorly documented. This is particularly true of tropical montane forest environments where limited observations indicate that the function of the soils of these ecosystems as sources and sinks is variable. We document and investigate the drivers of CH_4 fluxes from premontane, lower and upper montane forests experiencing a seasonal climate in south-eastern Peru between January 2011 and June 2013. These soils all functioned as net sinks for atmospheric CH_4 with wet and dry season means for net CH_4 flux and standard errors of -0.08 (0.13) and -0.20 (0.15) mg CH_4-C m^{-2} d^{-1} in premontane forest at 1070 - 1088 m asl, -0.97 (0.11) and -1.12 (0.13) mg CH_4-C m^{-2} d^{-1} in lower montane forest at 1532 - 1786 m asl and -1.04 (0.11) and -1.55 (0.13) mg CH_4-C m^{-2} d^{-1} in upper montane forest at 2811 - 2962 m asl. Variations between forest types were best explained by diffusional constraints, imposed by changes in water-filled pore space (WFPS), on the supply of CH_4 to high-affinity methanotrophic communities. Environmental controls on temporal variations varied within forest types. Most notably, despite a pronounced wet season between October and April, significant increase in net CH_4 fluxes were only observed in the upper montane forest. The decrease in net CH_4 flux with elevation in this study contradicts the pattern observed in the only other study of Andean montane forests in Ecuador. This suggests a better understanding of methanotrophic activity in the thick organic horizons typical of high elevation tropical forests is required.

3.2 Introduction

Methane (CH_4) is an important greenhouse gas (Cicerone and Oremland, 1988). Despite its importance, the comparison of satellite retrievals of the atmospheric concentra-

tion of CH_4 with source-sink inventories and process based models indicates that tropical landscapes are poorly characterised, likely reflecting historic imbalance in field observations, when compared to the northern hemisphere (Frankenberg et al., 2005; Bergamaschi et al., 2009; Bloom et al., 2010b). Soils play a key role in controlling atmospheric CH_4 concentrations with emissions from inundated wetland soils representing the largest natural source to the atmosphere, whilst, upland soils represents the only major biological sink (Denman et al., 2007). In this context, soils capable of acting as both globally significant sinks and sources for atmospheric CH_4, are of particular interest in refining the characterisation of CH_4 exchange across tropical landscapes (Dutaur and Verchot, 2007; Spahni et al., 2011).

The function of soil as source or sink for atmospheric CH_4 results from the balance between activity of aerobic methanotrophic bacteria and anaerobic methanogenic archaea (Le Mer and Roger, 2001). Upland soils are typically thought to act as a sink for atmospheric CH_4 as they support communities of high affinity methanotrophic bacteria which oxidise CH_4 at close to ambient concentrations using oxygen (O_2) concentrations in excess of 3 $\%$ as an electron acceptor (Bender and Conrad, 1992; Conrad, 1996; Teh et al., 2006). Following Michaelis-Menten kinetics where Km is greater than ambient CH_4 concentrations, variations in net flux are expected to arise from constraints on the rate of diffusion of CH_4 imposed by soil texture and slower liquid than gas-phase rates (Bender and Conrad, 1992; Smith et al., 2003). This reliance on diffusion indicates that high-affinity methanotrophs are likely to occupy well-connected pore spaces and as such uptake of atmospheric CH_4 is also sensitive to water stresses in drier soils (Von Fischer et al., 2009). Upland soils may also support anaerobic processes, concurrent with an oxic bulk soil matrix, within anoxic microsites (Sexstone et al., 1985; Conrad, 1996; Teh et al., 2005). Anoxic microsites form in response to constraints on the diffusion of O_2 into soil pores occluded by aggregates or saturation and biological uptake associated with autotrophic and aerobic heterotrophic respiration (Verchot et al., 2000; Teh and Silver, 2006). Under wet conditions and high O_2 demand, anaerobic metabolic activity can be significant and potentially lead to CH_4 emissions (Silver et al., 1999; Verchot et al., 2000; Davidson et al., 2004). In such situations, methanotrophy can consume the majority of CH_4 produced in situ through

the activity of low affinity communities utilising elevated concentrations close to the interface between anoxic and oxic niches (Conrad, 1996; Teh et al., 2005). In these circumstances, variations in net flux are expected to result from variations in the extent of conditions suitable for anaerobic and aerobic processes and competitive constraints on the supply of substrates to methanogenic communities associated with nitrate, iron and sulphate reduction (Silver et al., 1999; Chidthaisong and Conrad, 2000; Von Fischer and Hedin, 2007).

Tropical upland soils are estimated to account for approximately a third of the global atmospheric soil sink for CH_4 with nearly three quarters of this uptake occurring within forest environments (Dutaur and Verchot, 2007). However, in regions such South America tropical forests are expansive covering ~ 35 $\%$ of the continent and exhibit considerable spatial and temporal variability in soil-atmosphere CH_4 exchange (Eva et al., 2004; Keller et al., 1986; Steudler et al., 1996; Verchot et al., 2000; Davidson et al., 2004; Keller et al., 2005). The majority of studies have focussed on lowland forests, below 600 m asl, with variations explained by the influence of soil physical properties and moisture on uptake through the diffusion of CH_4 and emissions resulting from high aerobic activity in soils close to saturation (Verchot et al., 2000; Kiese et al., 2008; Dutaur and Verchot, 2007). In addition to lowland forest, South America has large areas of montane forest accounting for ~ 8 $\%$ of continental and ~ 25 $\%$ of Andean land-cover (Eva et al., 2004; Tovar et al., 2013). Observations of CH_4 cycling in the soils of montane forests are more limited than from lowland forests with published studies from Ecuador (Wolf et al., 2012), Brazil (Neto et al., 2011), Puerto Rico (Silver et al., 1999; Teh et al., 2005), Republic of Congo (Delmas et al., 1992), Indonesia (Purbopuspito et al., 2006; Ishizuka et al., 2005a), Australia (Kiese et al., 2008), China (Werner et al., 2006) and Hawaii (Schuur et al., 2001). As with their lowland counterparts, tropical montane and premontane forest soils principally act as a sink for atmospheric CH_4, however, the development of thick organic horizons may differentiate the activity of these soils at higher elevations (Delmas et al., 1992; Ishizuka et al., 2005a; Purbopuspito et al., 2006; Kiese et al., 2008; Neto et al., 2011; Wolf et al., 2012). For example, Wolf et al. (2012) indicate rates of CH_4 uptake decrease with elevation. In Ecuador spatial variations in CH_4 fluxes across premontane,

lower montane and upper montane forest soils were explained by negative correlations with factors such as CO_2 flux, ammonium concentration, and pH, indicating the uptake across these environments may be nitrogen limited and promoted by conditions generally favourable to microbial activity rather than parameters associated with diffusional constraints such as soil texture and soil moisture (Wolf et al., 2012). Similarly, temporal variations in CH_4 flux were not correlated with soil moisture within these forests or in an analogous study in Indonesia (Purbopuspito et al., 2006; Wolf et al., 2012). Whether this deviation from the expected controls on uptake reflects relatively small variations in soil moisture under the aseasonal climates of these regions is unclear. Whilst greater CH_4 fluxes during the wet than dry season are observed from a premontane Atlantic forest in Brazil, no correlation with soil moisture or temperature was identified (Neto et al., 2011). Similarly, no changes in net CH_4 flux across the dry to wet season transition were observed in a premontane forest in Northern Australia (Kiese et al., 2008). In contrast, studies from Puerto Rico and Hawaii indicate that tropical montane forests may represent regional hotspots for emissions, driven by increased methanogenic activity under lower soil O_2 concentrations, when compared to lower elevations (Silver et al., 1999; Schuur et al., 2001; Teh et al., 2005; Von Fischer and Hedin, 2007).

Here we present a study of soil-atmosphere CH_4 exchange, for the period January 2011 to June 2013, from Andean premontane, lower montane and upper montane forests in southeastern Peru which experience seasonal precipitation. The first year of this data has previously been reported by Teh et al. (2014) in a study of variations of non-CO_2 trace gas fluxes across montane forests and grasslands. This preliminary analysis of the data indicates that these forests act as a seasonably variable sink for atmospheric CH_4 and that differences in CH_4 flux across the forest - grassland transiton are driven by decreases in soil O_2 concentration. Here we aim to, 1) provide an assessment of variations in soil-atmosphere CH_4 exchange across the forests of this landscape and within forest types based on the complete dataset and, 2) investigate the drivers of CH_4 flux across these forests and within forest types. In this context our hypothesis is that soil O_2 concentration will explain best variations in net CH_4 flux.

3.3 Materials and methods

3.3.1 Study site

Three sites circa 1070 - 1088, 1532 - 1768 and 2811 - 2962 m above sea level (asl) in the southeastern Peruvian department of Cusco were selected as they represent montane and premontane forests typical of the eastern flank of the Andes (Figure 3.1 and 3.2). In this region premontane forests extend from ~ 600 to 1200 m asl, lower montane cloud forests from ~ 1200 to 2200 m asl and upper montane cloud forests from ~ 2200 m asl to the tree line at ~ 3400 m asl (Zimmermann et al., 2010b). The sites at 3030 and 1500 m asl representing upper and lower montane cloud forest, respectively, were proximal to the long term study sites of the Andes Biodiversity and Ecosystems Research Group (ABERG) elevational transect (Malhi et al., 2010). A new site was established in premontane forest at 1000 m asl owing to access issues at the original ABERG site at this elevation in 2010 and 2011. Site characteristics are summarised in Table 3.1.

Table 3.1: General characteristics of study locations. [1] mean annual temperature, [2] mean annual precipitation, [3] soil bulk density between 0 - 10 cm and [4] soil pH between 0 - 10 cm determined in soil: deionised water ratio of 1:5.

Study site	Hacienda Villa Carmen	San Pedro	Wayqecha
Forest type	premontane	lower montane	upper montane
Latitude (S)	12°53′43″	13°02′56″	13°11′24″
Longitude (W)	71°24′04″	71°32′13″	71°35′13″
Elevation (m asl)	1000	1500	3030
MAT[1] (°C)	23.4	18.8	12.5
MAP[2] (mm)	5318	2631	1706
Bulk density[3] ($\mathrm{g\,cm^{-3}}$)	0.38	0.19	0.10
pH[4]	3.4	3.4	3.9

The regional climate is seasonal with increased rainfall and slightly higher temperatures during the wet season between October and April, with these patterns becoming more pronounced at higher elevations (Figure 3.3). Precipitation and air temperature are greater at lower elevations with annual means at the upper and lower montane forest sites of, respectively, 1706 and 2631 mm for precipitation and 12.5 and 18.8 °C for temperature (Girardin et al., 2010). Mean annual precipitation and temperature at the

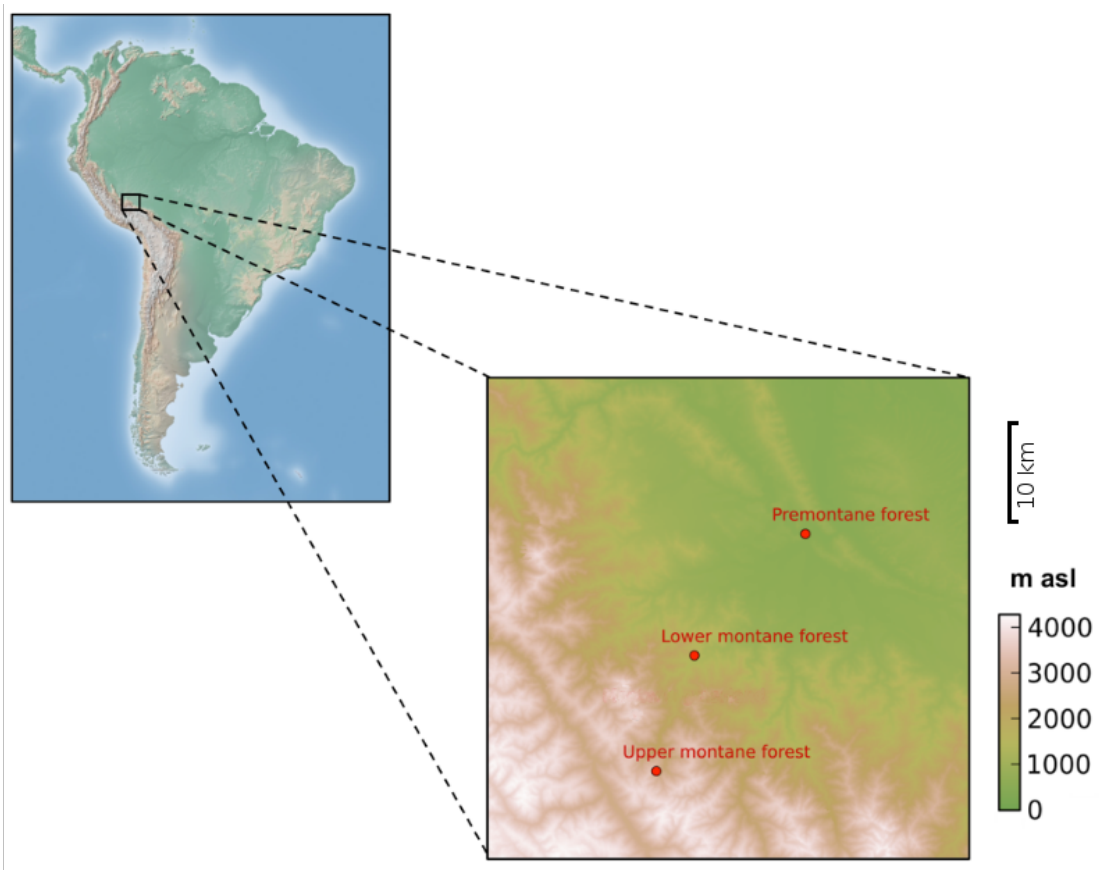

Figure 3.1: Location of the premontane forest site at Hacienda Villa Carmen, the lower montane cloud forest site at San Pedro and the upper montane forest cloud forest site at Wayqecha superimposed on a regional elevation map (image: M. Richards). The tree line in this area is ~ 3400 m asal.

premontane forest site is 5318 mm and 24.4 °C.

The soils of these forests vary with elevation, most notably the surficial soils in the montane forests typically consisted of thick organic horizons, ~ 20 cm deep in the upper and ~ 10 cm deep in the lower site, whilst, the surface soils in the premontane forest were principally mineral in origin (Zimmermann et al., 2009; Girardin et al., 2010). This pattern is reflected in the carbon contents of these soils with typical values for the upper 10 cm at the upper and lower montane forest sites of 40 - 50 % C and < 5 % C at the premontane forest site (Zimmermann et al., 2009). Similarly, bulk density in the upper montane, lower montane and premontane sites are 0.10, 0.19 and 0.38 $g\,cm^{-3}$, respectively. These soils are acidic with pH of 3.9, 3.4 and 3.4 at the upper montane, lower montane and premontane sites, respectively.

Soil-atmosphere CH_4 exchange for 2011 has previously been reported for these sites

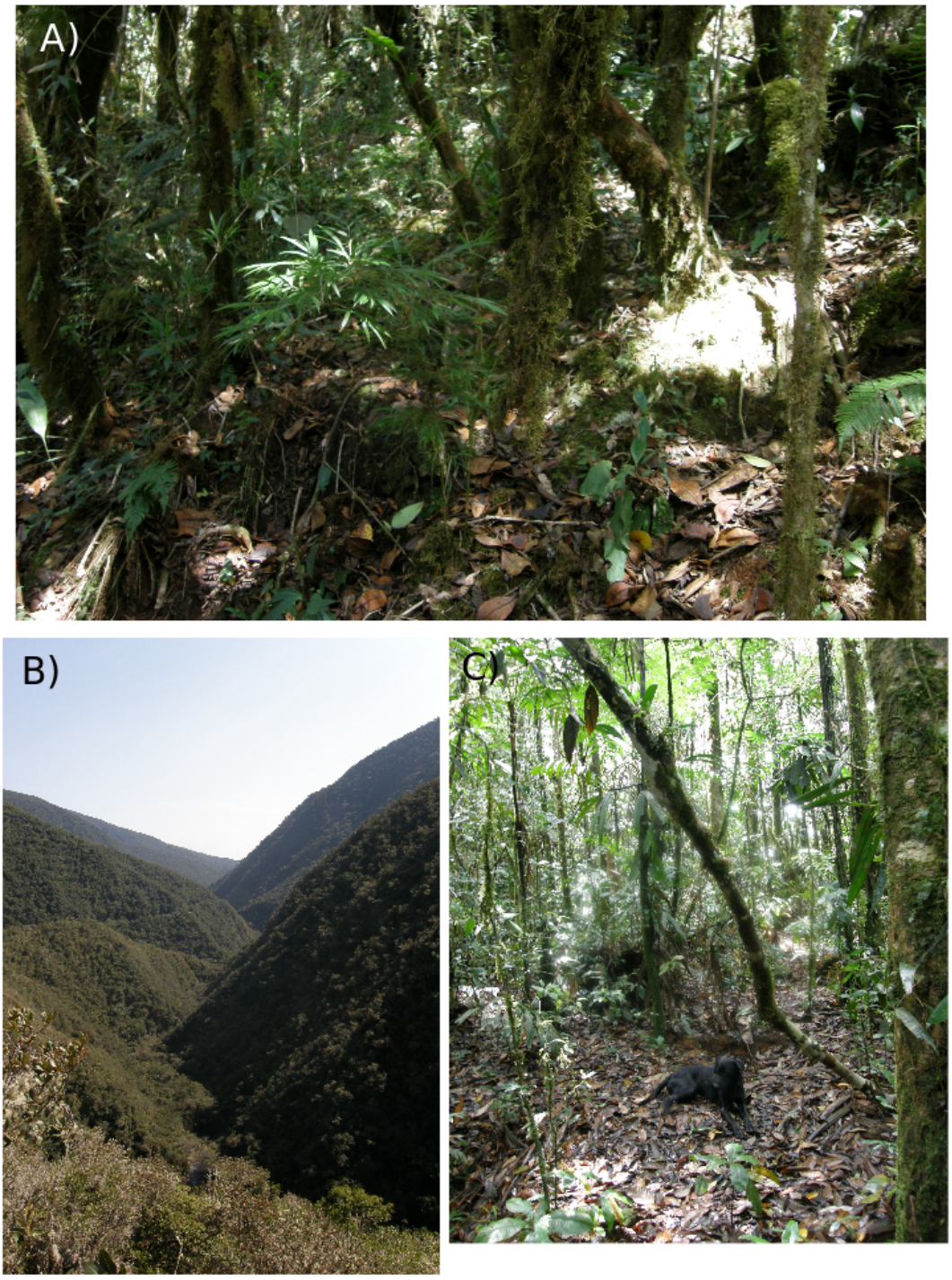

Figure 3.2: Examples of montane forests along the ABERG elevational transect: a) montane cloud forest with typical aboreal epiphytes at Wayqecha, b) montane forests growing on steep sleeps between 3000 and 1500 m asl in the Kosnipata valley and c) premontane forest at Hacienda Villa Carmen.

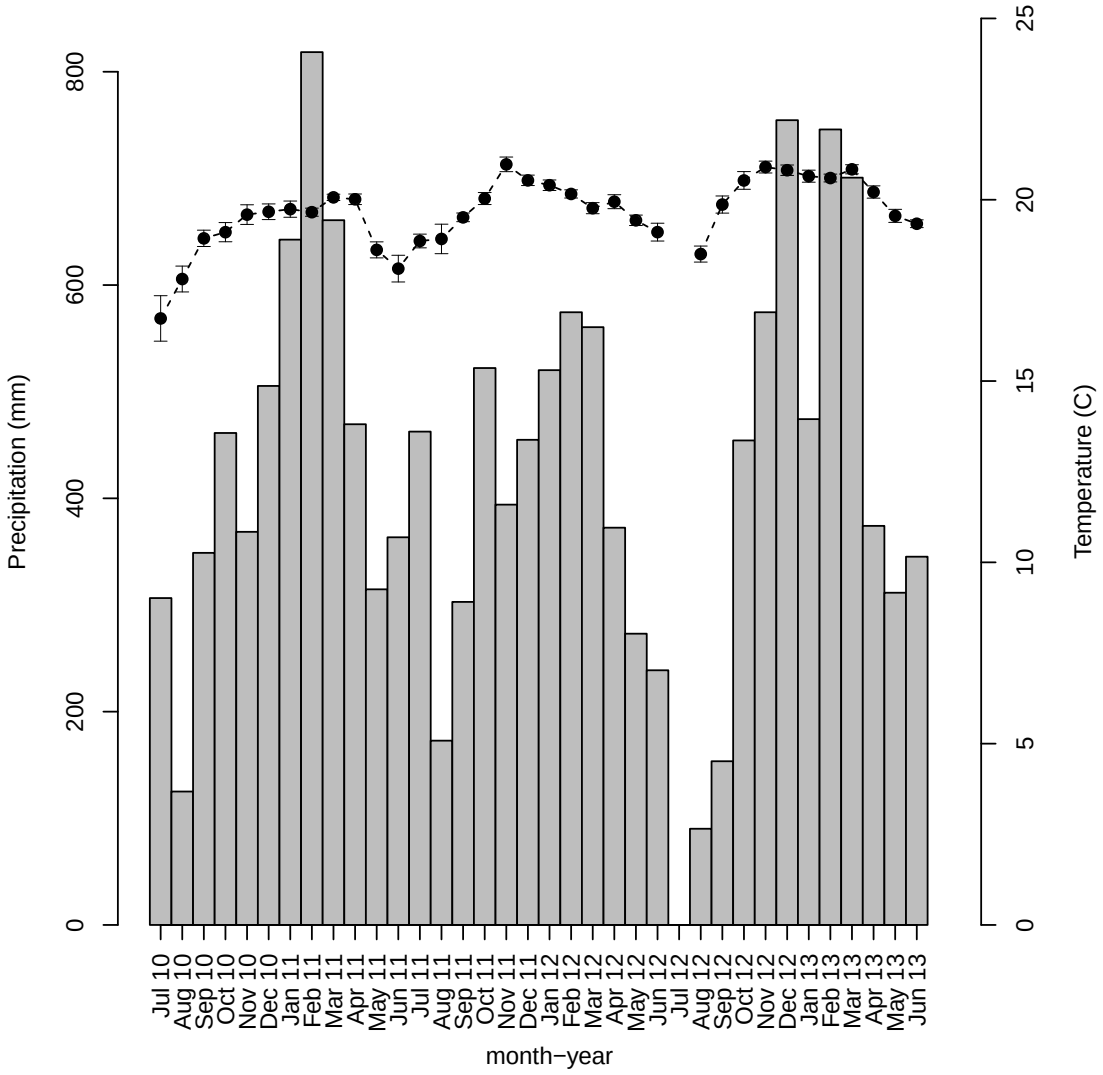

Figure 3.3: Total monthly precipitation and monthly mean day time temperature between July 2010 and June 2013 at 982 m asl (Chontachaca weather station: $13°01'26''$ S $71°28'04''$ W). Temperature error bars are standard error. No data was available for July 2012.

as part of a study investigating non-CO_2 trace gas fluxes along an Andean elevational transect (Teh et al., 2014). These measurements indicate that the CH_4 fluxes in the forests are small in comparison to source activity associated with wetlands in the montane grasslands found above the tree-line, with differences in CH_4 flux across this gradient best explained by a non-linear inverse relationship with O_2 concentration. In this context, the montane forests sites acted as sinks for atmospheric CH_4, whilst, the premontane forest had the potential to act as both a source or sink.

3.3.2 Sampling strategy

Within each forest type three plots of 20 by 20 m were established approximately two months prior to the start of measurements in an attempt to minimise the effects of disturbances involved with installing sampling equipment (Varner et al., 2003). Within forest types the distance between plots ranged from ~ 100 to 1000 m. The plots in the premontane forest were each situated on a ridge, slope and flat feature between elevations of 1070 to 1088 m asl. Similarly, the lower montane forest plots were established on ridge, slope and flat features between elevations of 1532 to 1768 m asl. In the upper montane forest two plots were situated on slopes and the third on a ridge at elevations between 2811 to 2962 m asl.

Within each plot five soil collars were installed to allow for measurements of soil-atmosphere gas exchange using a static flux chamber approach. Additionally, soil-gas equilibration chambers were buried at 10 cm adjacent to three collars in each plot to allow measurement of soil O_2 concentrations. Despite the three plots within each forest type broadly occurring within the same forest stand they were considered independent replicates of forest type as spatial correlations between net CH_4 fluxes in tropical forests are small (Ishizuka et al., 2005b; Purbopuspito et al., 2006).

Plots were visited monthly to measure soil-atmosphere gas exchange at each collar, soil moisture and temperature adjacent to each collar and soil O_2 concentration in each soil-gas equilibration chamber. In the upper and lower montane forests, measurements ran from January 2011 to June 2013. In the premontane forest measurements ran from July 2011 to June 2013. No data are available for the plots of this site in October or December of 2011 and February, July or December of 2012 as high river levels prevented access.

3.3.3 Soil-atmosphere gas exchange

Net soil-atmosphere fluxes of CH_4 and CO_2, were determined using a static chamber approach (Livingston and Hutchinston, 1995). Measurements were initiated by gen-

tly sealing cylindrical caps, using a section of inner-tube, to pre-installed soil collars to create a chamber of ~ 0.08 m^3 over a soil surface of ~ 0.03 m^2. Soil collars had a diameter of 20 cm and were inserted to a depth of ~ 5 cm. Each cap was equipped with a gas sampling port, air pressure equilibration port, and a 9 V computer fan (Pumpanen et al., 2004). Using a stopcock and 60 ml gas tight syringe, 20 ml gas samples were taken from the chambers at four discrete time steps over a period of ~ 30 min. Additionally, air temperature and atmospheric pressure were measured using a type K thermocouple (Omega Engineering Ltd.,UK.) and a Garmin GPSmap 60CSx (Garmin Ltd.,USA). Gas samples were stored in over-pressured, pre-evacuated 12 ml Exetainers (Labco Ltd., UK) and concentrations of CH_4 and CO_2 determined by gas chromatography.

Gas chromatography was conducted using a Thermo TRACE GC Ultra (Thermo Fisher Scientific Inc., USA) with a nitrogen carrier gas. A flame ionization detector (FID) and methanizer-FID were used to determine CH_4 and CO_2 concentrations, respectively. Analytes were separated using a Hayesep Q 100/200 column. The gas chromatograph was equipped with a 2 ml sample loop and oven temperature was 60 $^{\circ}\mathrm{C}$. Detector responses were calibrated using three or more, triplicated, certified gas standards (CK Gas Products Ltd., UK) and instrumental precision was deemed acceptable when coefficient of variances < 5 $\%$ were achieved. A custom-built auto-sampler (University of York, UK) was used to introduce gas samples directly from exetainers into the sample loop.

Fluxes, in $\mu\mathrm{l}\,\mathrm{l}^{-1}\,\mathrm{m}^{-2}\,\mathrm{s}^{-1}$, were calculated in R (R Core Team, 2013) using the HMR package (Pedersen, 2012). Following the criteria outlined by Pedersen et al. (2010), HMR or linear models were fitted to time-series of concentration in chamber headspaces. Significance was determined at the $p < 0.05$ level with emission and uptake indicated by positive and negative flux values, respectively. Non-significant fluxes were excluded from statistical analysis. Fluxes were converted from a concentration to amount basis reported in mg CH_4-C m^{-2} d^{-1} and g CO_2-C m^{-2} d^{-1}, following the ideal gas law, using measurements of air temperature and atmospheric pressure.

3.3.4 Soil environmental conditions

Soil O_2 concentration was measured from soil gas equilibration chambers, as previously described in 2.3.2.2, buried at 10 cm below the soil surface (Silver et al., 1999; Teh et al., 2005; Liptzin et al., 2011; Hall et al., 2013). Soil O_2 concentration was determined by withdrawing 40 ml of gas from a soil-gas equilibration chamber using a stopcock and gas tight syringe. The sample was then passed through the flow-through head of an MO-200 oxygen sensor (Apogee Instruments Inc., USA) into a second syringe. The O_2 reading was recorded, with a precision of 0.1 $\%$, and the gas sample reinjected into the soil-gas equilibration chamber from the second syringe. Prior to measurements the O_2 sensor was calibrated, as required, in field with air and the dead volumes of the sampling apparatus evacuated to minimise contamination of the soil gas sample by residual atmospheric air. Chambers had an interval volume of 50 ml and a surface area of 57 cm^2. Each consisted of a length of gas-permeable silicone rubber tubing (AP202/60 - 35 mm inner diameter by 1.5 mm wall, Advanced Polymers Ltd, UK) sealed at both ends with butyl rubber bungs. A suitable length of tygon tubing was passed through a hole in one of the bungs and capped with a stopcock to allow sampling at the surface. Chambers were encased in plastic mesh to protect the membrane during installation. Typical of similar designs, soil gas equilibration chambers were capable of equilibrating with the external atmosphere in less than 24 h (Holter, 1990; Jacinthe and Dick, 1996; Kammann et al., 2001).

Soil volumetric water content was determined from triplicate measurements at 10 cm below the surface using a ML2x ThetaProbe equipped with 12 cm rods (Delta-T Ltd., UK). Water-filled pore space (WFPS) was calculated from these data using plot estimates of porosity based on the bulk density, determined after drying for 24 h at 105 $^{\circ}\mathrm{C}$, of triplicate undisturbed samples from the upper 10 cm of soil at each plot. Soil temperature was determined from triplicate measurements at 10 cm using a type K penetration probe (Omega Engineering Ltd.,UK).

3.3.5 Statistical analyses

Statistical analysis was conducted on plot means of monthly measurements in R version 3.1.1 (R Core Team, 2013). Linear mixed effect models were used to test effects of the sampling structure and relationships between environmental variables as the dataset is unbalanced, with fewer measurements in the premontane forest, and nested within sampling month across forest types and in replicate plots within forest type (Pinheiro and Bates, 2000). In this respect, random intercept linear mixed effect models were computed using the NLME package and significance reported at $p < 0.05$ (Pinheiro et al., 2014). Model selection was made using likelihood ratios and the Akaike information criterion (AIC) and residuals were visually assessed for large deviations from assumptions of homogeneity and normality (Zuur et al., 2009). The pseudo-coefficients of determination for models are, calculated using the MuMIn package, reported with the marginal r^2 (mr^2) indicating the proportion of variance explained solely by fixed effects and the conditional r^2 (cr^2) indicating the proportion of variance explained by both fixed and random effects (Bartoń, 2014; Nakagawa and Schielzeth, 2013). Multiple collinearity of model fixed effects and their interactions were investigated by calculating variance inflation factors with collinearity assumed for values greater than 2 (Zuur et al., 2007). For interactions between environmental variables, variance inflation factors were > 30 and as such maximal models were specified with main effects only. The effect of forest type and season on monthly plot means of CH_4 flux, CO_2 flux, soil O_2 concentration, WFPS and soil temperature were investigated with forest type and season as fixed effects and sampling month and year as a random effect. Following model fits, multiple comparison of site and seasons was conducted in the multcomp package with Tukey contrasts (Hothorn et al., 2008). The effect of monthly plot means of CO_2 flux, soil O_2 concentration, WFPS, and soil temperature on monthly plot means of CH_4 flux across forest types was investigated with complete cases of CO_2 flux, soil O_2 concentration, WFPS and soil temperature as fixed effects and sampling month and year as a random effect. The effect of monthly plot means of CO_2 flux, soil O_2 concentration, WFPS, and soil temperature on month plot means of CH_4 flux within forest types was investigated with complete cases of CO_2 flux, soil O_2

concentration, WFPS and soil temperature as fixed effects and plot as a random effect.

3.4 Results

3.4.1 Variability in gas fluxes and soil environmental conditions

Fluxes of CH_4 were significantly influenced by forest type and its interaction with season, with larger fluxes at lower elevation and during the wet season (Table 3.2). All the forest types acted as a sink for atmospheric CH_4 with respective mean (standard error) net CH_4 fluxes during dry and wet season months of -1.55 (0.13) and -1.04 (0.11) mg CH_4-C m^{-2} d^{-1} in the upper montane forest, -1.12 (0.13) and -0.97 (0.11) mg CH_4-C m^{-2} d^{-1} in the lower montane forest and - 0.20 (0.15) and -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} in the premontane forest (Figure 3.4 a). During dry season months, net CH_4 fluxes varied significantly among forest types. During wet season months, net CH_4 fluxes from premontane forest were significantly larger than those from both the upper and lower montane forests. Within forest types, wet season CH_4 fluxes were only significantly larger than dry season fluxes in the upper montane forest. Uptake dominated soil-atmosphere exchange in the upper and lower montane forests with emissions accounting for 1 and 2 $\%$ of monthly mean CH_4 fluxes, respectively. Whilst net uptake was also evident in the premontane forest, the potential for source activity was considerably greater with emissions accounting for 29 $\%$ of observations.

Fluxes of CO_2 were significantly influenced by forest type and its interaction with season, with larger fluxes at lower elevation (Table 3.2). Fluxes of CO_2 during dry and wet season months were 2.88 (0.35) and 3.76 (0.26) g CO_2-C m^{-2} d^{-1} in the upper montane forest, 4.28 (0.35) and 3.86 (0.28) mg CO_2-C m^{-2} d^{-1} in the lower montane forest and 5.13 (0.39) and 4.88 (0.36) g CO_2-C m^{-2} d^{-1} in the premontane forest (Figure 3.4 b). Dry season CO_2 fluxes were significantly smaller in the upper montane forest than both the lower montane and premontane forests, whilst, during the wet season CO_2 fluxes from premontane forest were significantly larger than those from both the upper and lower montane forests. Within forest types, there were no significant seasonal

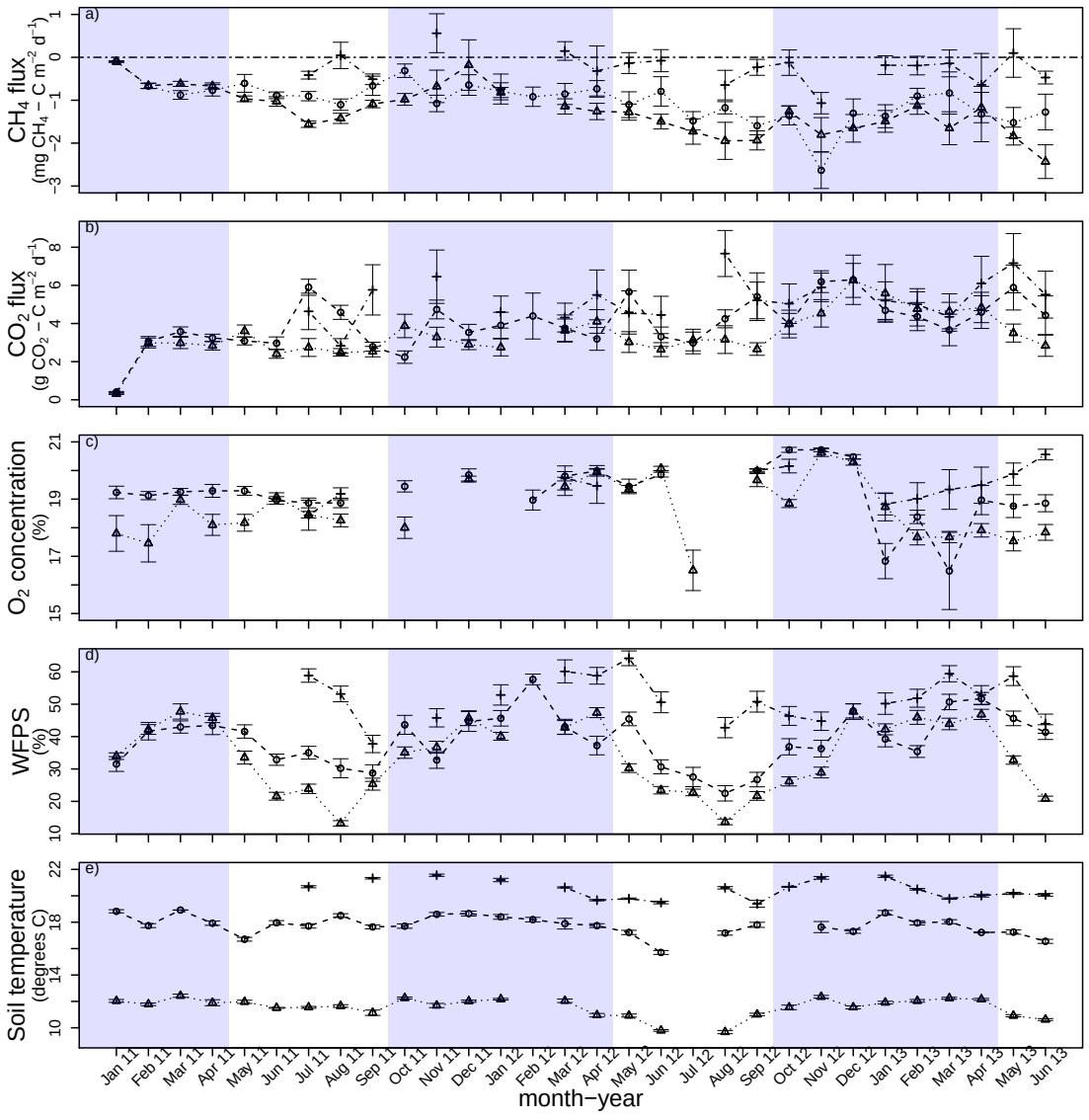

Figure 3.4: Monthly forest type (△ upper montane forest, ∘ lower montane forest, + premontane forest) means and standard error bars of a) net CH_4 flux (n = 15 / site), b) net CO_2 flux (n = 15 / site), c) soil O_2 concentration (n = 9 / site), d) WFPS (n = 15 / site) and c) soil temperature (n = 15 / site). Shading indicates wet season of October - April.

Table 3.2: Forest type means and standard errors for aggregated dry (May - September, n = 8, n = 11 for lower and upper montane) and wet (October - April: n = 10 for premontane, n = 17 for lower and upper montane) season months. Capital letters indicate significant differences among forest types within season and lower case letters indicate significant differences between season within forest types.

	Forest type	Premontane	Lower montane	Upper montane
dry	Net CH_4 flux	-0.20 (0.15) Aa	-1.12 (0.13) Ba	-1.55 (0.13) Cb
wet	(mg CH_4-C m^{-2} d^{-1})	-0.08 (0.013) Aa	-0.97 (0.11) Ba	-1.04 (0.11) Ba
dry	Net CO_2 flux	5.13 (0.39) Aa	4.28 (0.35) Aa	2.88 (0.35) Ba
wet	(g CO_2-C m^{-2} d^{-1})	4.88 (0.36) Aa	3.86 (0.28) Ba	3.76 (0.29) Ba
dry	O_2 concentration	19.7 (0.3) Aa	19.1 (0.3) ABa	18.6 (0.3) Ba
wet	(%)	19.8 (0.2) Aa	19.2 (0.2) ABa	18.8 (0.2) Ba
dry	WFPS	51.5 (2.1) Aa	34.0 (2.0) Bb	23.6 (2.0) Cb
wet	(%)	53.4 (1.9) Aa	42.3 (1.6) Ba	41.8 (1.6) Ba
dry	Soil temperature	20.4 (0.2) Aa	17.3 (0.2) Bb	11.0 (0.2) Cb
wet	(°C)	20.7 (0.2) Aa	18.1 (0.1) Ba	11.9 (0.1) Ca

differences in CO_2 flux.

Soil O_2 concentration was significantly influenced by forest type, with greater concentrations at lower elevation (Table 3.2). Soil O_2 concentrations during dry and wet season months of 18.6 (0.3) and 18.8 (0.2) % in the upper montane forest, 19.1 (0.3) and 19.2 (0.2) % in the lower montane forest and 19.7 (0.3) and 19.8 (0.3) % in the premontane forest (Figure 3.4 c). In both wet and dry season soil O_2 concentration was significantly smaller in the upper montane than the premontane forest. Within forest types, there were no significant seasonal differences in soil O_2 concentration.

WFPS was significantly influenced by forest type and its interaction with season, with greater saturation at lower elevation and during the wet season (Table 3.2). Mean WFPS during dry and wet season months was 23.6 (2.0) and 41.8 (1.6) % in the upper montane forest, 34.0 (2.0) and 42.3 (1.1) % in the lower montane forest and 51.5 (2.1) and 53.4 (1.9) % in the premontane forest (Figure 3.4 d). Dry season WFPS was significantly different between all forest types, whilst, during the wet season WFPS in the premontane forest was significantly greater than those from both the upper and lower montane forests. Within forest types, WFPS was significantly greater for wet than dry season for both the upper and lower montane forests.

Soil temperature was significantly influenced by forest type and its interaction with

season. Soil temperatures were greater at lower elevation and during the wet season (Table 3.2). Mean soil temperature during dry and wet season months was 11.0 (0.2) and 11.9 (0.1) ℃ in the upper montane forest, 17.3 (0.2) and 18.1 (0.1) ℃ in the lower montane forest and 20.4 (0.2) and 20.7 (0.2) ℃ in the premontane forest (Figure 3.4 e). In both seasons, soil temperatures were significantly different between all forest types. Within forest types, soil temperatures were significantly greater during the wet than dry season in both the upper and lower montane forests.

3.4.2 Drivers of variability in net CH_4 flux

Across forest sites and the measurement period, the minimal significant model explaining variations in CH_4 flux was a negative relationship with CO_2 flux and positive relationships with both WFPS and soil temperature (AIC = 333, mr^2 = 0.32, cr^2 = 0.37). Of these significant effects, the strongest relationship is found with WFPS (AIC = 358, mr^2 = 0.22, cr^2 = 0.42), followed by soil temperature (AIC = 357, mr^2 = 0.18, cr^2 = 0.36), whilst the influence of CO_2 is comparatively weak (AIC = 396, mr^2 = 0.01, cr^2 = 0.10). In this respect, differences in CH_4 flux among the forest types are best explained by a positive relationship with WFPS (Figure 3.5).

Within the premontane forest, no significant relationships between variations in CH_4 flux and CO_2 flux, O_2 concentration, WFPS or soil temperature across the measurement period were identified (AIC = 47, mr^2 = 0.00, cr^2 = 0.10). Within the lower montane forest, the minimal significant model explaining variations in CH_4 flux across the measurement period was a negative relationship with CO_2 flux (AIC = 147, mr^2 = 0.18, cr^2 = 0.18) . Within the upper montane forest, the minimal significant model explaining variations in CH_4 flux across the measurement period was a negative relationship with CO_2 flux and positive relationships with both WFPS and soil temperature (AIC = 107, mr^2 = 0.44, cr^2 = 0.55). Of these significant effects, the strongest relationship is found with soil temperature (AIC = 137, mr^2 = 0.14, cr^2 = 0.29), followed by CO_2 flux (AIC = 139, mr^2 = 0.13, cr^2 = 0.22) and WFPS (AIC = 141, mr^2 = 0.09, cr^2 = 0.24).

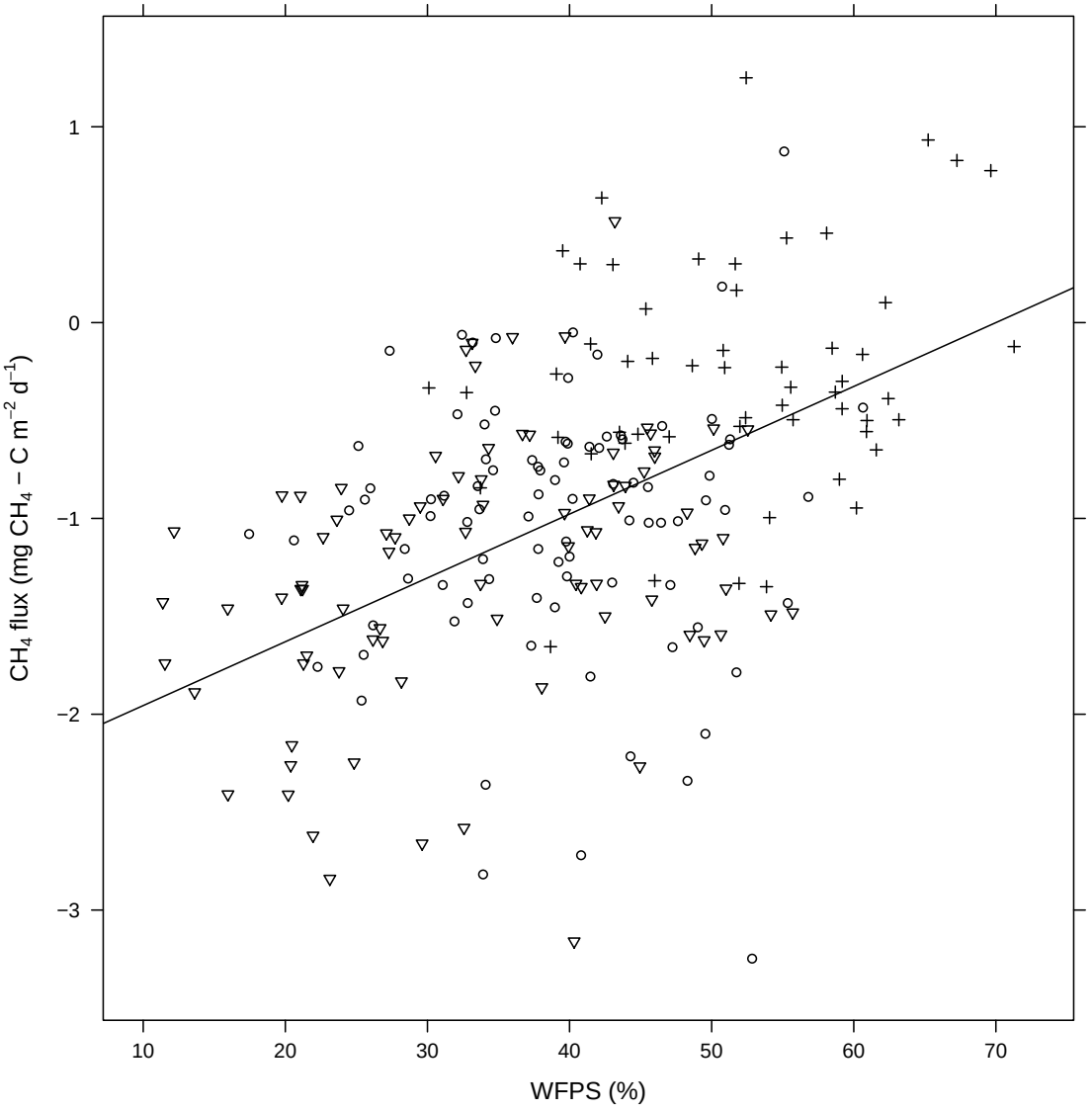

Figure 3.5: Relationship between monthly forest type (△ upper montane forest, ○ lower montane forest, + premontane forest) means of net CH_4 flux and WFPS (n = 174 in 25 groups). The line is the slope of the linear random effects model (y = fixed effects | random effects): net CH_4 flux = 0.03 × WFPS - 2.28 | 0.35 × month/year, AIC = 358, mr^2 = 0.22, cr^2 = 0.42.

3.5 Discussion

3.5.1 Uptake of CH_4 in tropical Andean forests of southern Peru

In this study premontane, lower and upper montane forests in the southern tropical Andes of Peru principally acted as sinks for atmospheric CH_4. Mean net CH_4 fluxes from these soils ranged from -1.55 to -0.08 mg CH_4-C m^{-2} d^{-1}, indicating that soil-atmosphere CH_4 exchange in these forests is comparable to similar environments globally (Table 3.3). Reported net CH_4 fluxes for tropical forest soils above 600 m asl range from -1.62 to -0.16 mg CH_4-C m^{-2} d^{-1} for the northern Andes in Ecuador (Wolf et al., 2012), -0.91 to -0.27 mg CH_4-C m^{-2} d^{-1} for central Sumatra and Sulawesi in Indonesia (Ishizuka et al., 2005a; Purbopuspito et al., 2006), -0.12 to -0.02 mg CH_4-C m^{-2} d^{-1} for Mayombe highlands in the Republic of Congo (Delmas et al., 1992), -0.66 mg CH_4-C m^{-2} d^{-1} for a tableland in northern Australia (Kiese et al., 2008), -0.53 mg CH_4-C m^{-2} d^{-1} in China (Werner et al., 2006) and -1.2 mg CH_4-C m^{-2} d^{-1} for Atlantic forest in Brazil (Neto et al., 2011). Similarly, soil-atmosphere CH_4 exchange rates for lowland tropical forests in South America have been reported in the range of -1.35 to -0.06 mg CH_4-C m^{-2} d^{-1} (Keller et al., 1986; Steudler et al., 1996; Fernandes et al., 2002; Verchot et al., 2000; Keller et al., 2005; Davidson et al., 2004, 2008; Neto et al., 2011).

During wet season months net CH_4 fluxes were larger, indicating weaker uptake, than during dry season months in all forest types in this study, however, this difference was only significant in the upper montane forest with means of -1.04 (0.11) mg CH_4-C m^{-2} d^{-1} for months of October through April and -1.55 (0.13) mg CH_4-C m^{-2} d^{-1} for the months of May through September. This temporal variability contrasts with observations from montane forests in Ecuador and Indonesia with aseasonal climates, but reflects the behaviour of lowland tropical forests where the direction and magnitude of CH_4 fluxes is modulated by precipitation patterns (Verchot et al., 2000; Keller et al., 2005; Purbopuspito et al., 2006; Davidson et al., 2008; Wolf et al., 2012). We did not observe notable spatial or temporal hotspots of CH_4 emission in these forests, contrasting with observations from regions such as Puerto Rico where Silver et al. (1999)

Table 3.3: Characteristics and annual mean (standard error) net CH_4 fluxes reported forests above 600 m asl. [1] mean annual precipitation, [2] mean annual temperature and [3] organic horizon thickness. NA indicates no data were published. [a] this study, [b] (Wolf et al., 2012), [c] (Purbopuspito et al., 2006), [d] (Ishizuka et al., 2005a), [e] (Silver et al., 1999), [f] (Delmas et al., 1992), [g] (Neto et al., 2011) and [h] (Kiese et al., 2008).

Country -	Elevation (m asal)	Forest type (montane)	MAP[1] (mm)	MAT[2] (°C)	O-horizon[3] (cm)	Net CH_4 flux (mg CH_4-C m^{-2} d^{-1})
Peru[a]	1070 - 1088	pre	5318	23.4	< 5	-0.14 (0.10)
"	1532 - 1768	lower	2631	18.8	~ 10	-1.05 (0.90)
"	2811 - 2962	upper	1706	12.5	~ 20	-1.30 (0.09)
Ecuador[b]	990 - 1200	pre	2230	19.4	2.5 - 6.5	-1.54 (0.14)
"	1800 - 2100	lower	1950	15.7	4.0 - 24	-0.85 (0.28)
"	2800 - 3000	upper	4500	9.4	6.6 - 22.2	-0.29 (0.12)
Indonesia[c]	1190	pre	1500	22.5	0	-0.67 (0.25)
"	1800	lower	NA	18.3	15 - 25	-0.91 (0.18)
"	2470	upper	NA	14.6	10 - 20	-0.40 (0.05)
Indonesia[d]	870	NA	NA	NA	NA	-0.27 (0.57)
"	1540	NA	NA	NA	NA	-0.87 (1.21)
Puerto Rico[e]	750	pre	4500	21	NA	0.24 (0.18)
"	1050	lower	5000	19	NA	73.3 (37.3)
Congo[f]	< 900	NA	1500	NA	NA	-0.12
"	< 900	NA	1500	NA	NA	-0.02
Brazil[g]	1000	NA	2300	19.1	NA	-1.20 (0.08)
Australia[h]	700 - 900	NA	1594	20.9	NA	-0.66 (0.01)

report mean net CH_4 fluxes of 0.24 (0.18) to 73.25 (37.34) mg CH_4-C m^{-2} d^{-1} (Schuur et al., 2001; Teh et al., 2005). Indeed, source activity in the upper and lower montane forests of Peru represented only 1 - 2 % of fluxes. Emissions were more prevalent in the premontane forest, accounting for 29 % of fluxes, suggesting that source hotspots are possible in these soils but were not captured by our sampling strategy (Delmas et al., 1992; Silver et al., 1999; Davidson et al., 2004).

3.5.2 Biogeochemical controls on net CH_4 fluxes in tropical Andean forests of southern Peru

Among forest types, net CH_4 uptake increased with elevation. For example, net CH_4 flux rates during the dry season were -0.20 (0.15) mg CH_4-C m^{-2} d^{-1} in the premontane forest, -1.2 (0.13) mg CH_4-C m^{-2} d^{-1} in the lower montane forest and -1.55 (0.13) mg CH_4-C m^{-2} d^{-1} in the upper montane forest. A similar pattern was observed for

wet season months with marginal uptake of -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} in the premontane forest, but more similarity in uptake for the lower and upper montane forests of - 0.97 (0.11) and -1.04 (0.11) mg CH_4-C m^{-2} d^{-1}. These among forest type differences in CH_4 fluxes were best explained by positive relationships with WFPS and soil temperature and a negative relationship with CO_2 flux. In this respect, we reject our hypothesis as no relationship was found between net CH_4 fluxes and soil O_2 concentration, which ranged from 18.6 to 19.8 $\%$, among forest types. The predominance of CH_4 uptake and the lack of evidence for widespread anoxia indicates that CH_4 cycling within these soils is dominated by the activity of high affinity methanotrophs. Decreases in WFPS with elevation best explained among forest type differences in CH_4 fluxes with respective means for premontane, lower and upper montane forest of 51.5 (2.1), 34.0 (2.0), 23.6 (2.0) $\%$ during dry season months and 53.4 (1.9), 42.3 (1.6) and 41.8 (1.6) $\%$ for wet season months (Figure 3.5). In this context the positive relationship between net CH_4 fluxes and WFPS across forest types conforms to the expectation that high-afffinity methanotrophy is limited by CH_4 supply in response to diffusional constraints imposed by the interaction between soil structure and water content (Smith et al., 2003; Curry, 2007; Von Fischer et al., 2009). Notably this relationship appears to be strongest, reflecting greater dissimilarity in WFPS for these periods and imbalance in the number of observations among forest types, for the upper and lower montane forest plots during dry season months. The importance of such spatial relationships between soil-atmosphere CH_4 exchange and WFPS have previously been highlighted for lowland tropical soils but not in studies across montane forests where gravimetric water contents rather than WFPS have previously been reported (Verchot et al., 2000; Purbopuspito et al., 2006; Wolf et al., 2012). The positive relationship identified between net CH_4 flux and soil temperature is difficult to explain in terms of the expected controls on methanotrophy as rates of microbial activity would be expected to increase rather than decrease in response to increases in temperature. This situation may reflect the coincidence of both greater WFPS and soil temperature at lower elevation (Neto et al., 2011). Such a situation may be supported by the weak negative relationship between CO_2 flux and net CH_4 flux indicating that uptake is greater under conditions that are favourable to the general activity of aerobic microbes (Purbopuspito et al.,

2006; Wolf et al., 2012). Alternatively this relationship may reflect the temperature sensitivity of CH_4 dissolution into the microbial films of methanotrophic communities.

The controls on temporal variations in net CH_4 flux differed within forest types. As with comparison among forest types, we find no evidence for soil O_2 concentration as a factor in determining variability in net CH_4 fluxes. In the premontane forest no drivers of net CH_4 flux were identifiable reflecting the limited number of observations from this site and the lack of significant seasonality identified for net CH_4 fluxes or any of the measured parameters (Wolf et al., 2012; Purbopuspito et al., 2006; Neto et al., 2011). In the lower montane forest increases in net CH_4 flux, or decreases in uptake rate, were related to decreases in CO_2 flux indicating that conditions favourable for methnaotrophy may be similar to those for general soil respiration (Purbopuspito et al., 2006; Wolf et al., 2012). Significant differences in WFPS between wet and dry season months was not reflected in net CH_4 fluxes from these soils suggesting these variations were not great enough to sufficiently limit diffusion of CH_4 as to constrain uptake rates. In upper montane forest net CH_4 fluxes were positively correlated with soil temperature and WFPS and negatively correlated with CO_2 fluxes. Whilst the relationships with WFPS and CO_2 flux are in keeping with observed controls on methanotrophy in other systems, the positive relationship with soil temperature is less intuitive. Similarly to covariance between WFPS and soil temperature with elevation, this situation in the upper montane forest may reflect greater temperatures during the wet season months resulting from greater night time cloud insulation or constraints on the dissolution of CH_4.

3.5.3 The role of elevation in soil CH_4 cycling in global tropical montane forests

With the exception of evidence of hotspots of source activity in some montane forests, rates of CH_4 uptake in tropical montane forest soils are broadly comparable across similar environments globally. However, within montane regions the relationship between soil-atmosphere CH_4 exchange and elevation is poorly constrained with contrasting patterns reported in Peru by this study, Ecuador by Wolf et al. (2012) and Indonesia by

Purbopuspito et al. (2006). The relationship between elevation and edaphic conditions is broadly similar, with premontane, lower and upper montane forest ecotones occurring within similar elevational bands, for these elevational transects (Foster, 2001). In this respect, the soils of these montane forests are differentiated from premontane and lowland forest soils by the presence of thick organic horizons at the surface. Despite this similarity, inverse relationships between net CH_4 flux and elevation are identified for the soils of Peru and Ecuador with greatest uptake in the upper montane and premontane soils, respectively (Table 3.3). Furthermore, uptake is greatest in the lower montane soils in Indonesia. From these contrasts it is possible to suggest that temperature, as might be expected, is not an important constraint on CH_4 uptake in montane forest soils despite previously identified correlations in this study and Ecuador (Wolf et al., 2012). Speculation as to the causes of these contrasts is difficult due to discrepancies in collection and publication of environmental parameters; for example, inverse relationships in Peru and Ecuador may reflect differences observed in precipitation regimes but comparison in terms of WFPS is not possible without estimates of porosity. Similarly, indications that CH_4 uptake in the Ecuadorian soils may be nitrogen limited with a positive relationship with ammonium are not replicated in Indonesia where no significant relationship with ammonium is found (Purbopuspito et al., 2006; Wolf et al., 2012). Indeed, there is evidence to suggest that the Peruvian forests of this study may broadly become nitrogen limited with elevation indicating further potential contrasts (Fisher et al., 2013). Wolf et al. (2012) highlight that organic horizons of these montane forests, unlike those of temperature forests, are active zones of methanotrophy. In this respect, it is likely that a better understanding of the edaphic controls on methanotrophy in such soils is required to reconcile differences in net CH_4 fluxes across these montane landscapes.

3.6 Conclusions

The findings of this study suggest that the premontane, lower and upper montane forests of southeastern Peru principally act as sinks for atmospheric CH_4. Uptake rates in these soils are comparable to activity observed globally for both montane and low-

land tropical forests. Uptake rates were greatest in the upper montane forest and lowest in the premontane forest. We find that across the landscape these soils are predominantly oxic and the soil CH_4 dominated by the activity of high affinity methanotrophy. In this regard, variations in WFPS reflecting contraints on the diffusional ingress of CH_4 from the atmosphere best explained the variation in net CH_4 flux among forest types. Despite the seasonality of precipitation in this region, significant wet season increases in net CH_4 fluxes were only identified in the upper montane forest soils. The increase in CH_4 uptake with elevation contrasts with that previously reported for similar environments in Ecuador and Indonesia suggesting that an improved understanding of the controls on methanotrophy in the organic horizons of montane forest soils is required.

Chapter 4

Methane cycling across a humid puna ecosystem in the southern Peruvian Andes

Sam Jones[1], Torsten Diem[3], Lidia Huaraca Quispe[2], Nelson Cahuana Valderrama [2], Fernando Hanceo Pacha[2], Jimmy Chambi Paucar[2], Beisit Puma Vilca[2], Charol Quispe Quispe[2], Patrick Meir[1] and Yit Arn Teh[3]

[1]School of GeoSciences, University of Edinburgh, Edinburgh, United Kingdom, [2]Universidad Nacional de San Antonio Abad del Cusco, Cusco, Peru, [3]Institute of Biological and Environmental Sciences, University of Aberdeen, United Kingdom

This chapter reports soil-atmosphere CH_4 exchange and soil environmental conditions for a humid puna, or montane grassland, ecosystem in Peruvian Andes. Long-term measurements were collected as part of a NERC grant entitled 'Are tropical uplands regional hotspots for methane and nitrous oxide?'. Here we report the full period of measurements, January 2010 to June 2013, for this data set. Additionally intensive seasonal campaigns during the wet season of 2011 and dry season 2012 and a laboratory incubation experiment are considered. Supplementary data for the field measurements can be found in Appendix D and the incubation experiment in Appendix A. The first year of the long-term and the wet season intensive campaign data has previously been published as Teh et al. (2014). A copy of this publication can be found in Appendix E. Contributions to this chapter have been made by Sam Jones, Torsten

Diem, Lidia Huaraca Quispe,Nelson Cahuana Valderrama, Fernando Hanceo Pacha, Jimmy Chambi Paucar, Beisit Puma Vilca, Charol Quispe Quispe, Patrick Meir and Yit Arn Teh. Yit Arn Teh and Patrick Meir won the funding and outlined the basis of the experimental design for the long-term measurements. Additionally, they provided advice in all stages of the work. Torsten Diem orchestrated the installation of long-term measurement plots and monthly data collection with the assistance of Lidia Huaraca Quispe and Sam Jones. Sam Jones designed and constructed the soil-gas equilibration chambers and developed the protocol for field measurements of soil oxygen concentration. Gas chromatography and calculation of net flux rates for the long-term measurements was conducted by Torsten Diem. Sam Jones designed and implemented the sampling approach, measurements and laboratory analysis for the intensive campaigns and incubations. Fernando Hanceo Pacha and Jimmy Chambi Paucar provided assistance collecting field measurements during 2011 campaign and Beisit Puma Vilca and Charol Quispe Quispe provided assistance during the 2012 campaign. Nelson Cahuana Valderrama identified plants in the vegetation survey. Sam Jones conducted all statistical analysis and writing.

4.1 Abstract

Discrepancies between top-down and bottom-up estimates of the tropical South American atmospheric CH_4 budget indicate that sources of CH_4 in this region have not been fully characterised. In this context we report soil-atmosphere CH_4 exchange and associated environmental variables from a humid puna, montane grassland, ecosystem in the Southeastern Andes of Peru. We documented soil-atmosphere CH_4 exchange across a grassland-wetland complex through long-term measurements conducted monthly from January 2011 through June 2013 and intensive seasonal campaigns from the 12^{th} through 22^{nd} of November 2011 and 12^{th} through 21^{st} of August 2012. We aimed to investigate the spatial controls on net CH_4 fluxes both across and within landscape features by characterising both edaphic and environmental conditions. We investigated the constraints on in vitro rates of net and gross CH_4 cycling rates using an isotope pool dilution technique. We identify considerable source poten-

tial for this landscape, particularly from wetland hotspots and during the wet season, with mean net CH_4 fluxes and standard errors from long-term measurement plots during dry and wet season months of -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} on the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} on the slope, -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} in the depression and 24.65 (10.70) and 181.74 (36.35) mg CH_4-C m^{-2} d^{-1} from the hollow. Characterisation of edaphic conditions across this landscape confirms emissions from both upland and wetland soils should be addressed when considering the regional influence of such ecosystems. Variations in net CH_4 flux across the landscape, from uptake to emission, were best explained by soil O_2 concentration reflecting the balance between methanogenic and methanotrophic activity. Incubations indicate that methanogenic activity is ubiquitous in these soils despite oxic conditions. Positive correlations between gross production rates, gross consumption rates and total soil C mineralisation indicate that methanogenic activity across the landscape is dependent on the total availability of C to microbial communities and that methanotrophs are capable of consuming significant portions of endogenous methane.

4.2 Introduction

The availability of satellite retrievals of atmospheric methane (CH_4) concentration has fueled recent interest in understanding variability in the global atmospheric budget of this radiatively important greenhouse gas (Frankenberg et al., 2005; Bergamaschi et al., 2009; Frankenberg et al., 2011). Such studies have re-confirmed the findings of previous inverse modelling simulations using airborne or ground sampling networks that the tropics are a larger source of CH_4 to the atmosphere than previously thought and that current bottom-up source sink inventories based on scaling of field observation or process-models poorly characterise landscape-atmosphere exchange in these regions (Mikaloff Fletcher et al., 2004a,b; Bloom et al., 2010b). In this respect, there has been a drive to better constrain not only the dynamics of traditional wetland sources, such as tropical swamp and seasonally inundated floodplain forests (Melack et al., 2004; Bloom et al., 2010b; Ringeval et al., 2010) but also less well understood potential sources of CH_4 to the atmosphere such as emissions from wet upland soils (Teh

et al., 2005; Megonigal and Guenther, 2008; Spahni et al., 2011), trees as both sources and conduits (Terazawa et al., 2007; Gauci et al., 2010; Covey et al., 2012), abiotic degradation of foliar pectin (Keppler et al., 2006; Bowling et al., 2009; Bloom et al., 2010a) and uncharacterised wetland environments in upland settings like the leaf axes of canopy epiphytes in neo-tropical forests (Martinson et al., 2010). In this context, the highlands of the tropical Andes are of particular interest as the presence of organic rich mineral soils and peatlands in high altitude montane ecosystems potentially represent a poorly documented but significant component of the tropical South American CH_4 budget (Wania et al., 2009; Page et al., 2011; Teh et al., 2014).

The tropical Andes, extending from latitudes of 11° N to 23° S and elevations of 600 to 6962 m asl (above sea level), represents a hotspot for biodiversity and endemism covering some $1.27 \times 10^{6}\,\mathrm{km}^{2}$ (Myers et al., 2000; Tovar et al., 2013). Between tree and permanent snow-lines there are diverse grass and shrub dominated ecosystems, known variously depending on species composition, from Venezuela to Bolivia as paramo, jalca and puna. These environments are extensive accounting for ~ 37 $\%$ of tropical Andean land-cover and range from xeric to humid, shrub and grassland, through to wet mosaics of upland grasslands and wetland bogs and lakes (Josse et al., 2009a,b, 2011; Tovar et al., 2013). The broad geographical distribution of these ecosystems relates to the orogenic history of the Andes and climatic conditions imposed by latitude and orography (Luteyn and Churchill, 1999; Josse et al., 2009a,b, 2011). The northern Andes from its limit in the Sierra de Peria and Cordillera de Merida of Venezuela through to the intersected western, central and eastern ranges of Colombia, Ecuador and northern Peru, typically experiences abundant, aseasonal precipitation (Josse et al., 2011). This climate supports wet paramo grasslands emerging above evergreen montane tropical forests on both the western and eastern Andean slopes (Josse et al., 2009a,b). Southwards in the central tropical Andes, the western and eastern ranges merge to eventually form the expansive highland plains of the Altiplano in Southern Peru and Bolivia (Josse et al., 2011). These environments are drier than the Northern Andes as seasonality in precipitation becomes more pronounced in the south. Here the wet paramos of the Northern Andes transition to drier, humid puna grasslands bounded, owing to the rain-shadow effect associated with moist air moving westwards from the

Amazon basin, by evergreen montane tropical forest on the steep eastern, Amazonian flank and seasonally dry tropical montane forest and xeric puna grass and shrubland on the western, Pacific flank (Josse et al., 2009a,b). Towards the tropical limits of the Andes, these xeric ecosystems spread eastward as seasonality and the influence of orography become more pronounced across the broadening Bolivian Altiplano (Josse et al., 2009a,b). Particularly in the paramo and north and eastern extents of the humid puna these ecosystems are characterised by rolling tussock grasslands with wet organic rich mineral soils and topographically constrained lakes and peat forming wetlands dominated by mosses and rushes or cushion plants (Miller and Birkeland, 1992; Hofstede, 1995; Zimmermann et al., 2010b). Limited field measurements indicate that such soils, as in analogous environments globally, can function as a source of CH_4 to the atmosphere and as such an improved understanding of soil-atmosphere CH_4 exchange in these ecosystems is required (Spahni et al., 2011; Turetsky et al., 2014; Teh et al., 2014). In this respect, documenting and understanding the controls on CH_4 cycling across mesoscale, delineating transitions between upland grassland and wetland environments, and microscale, reflecting variability within environments, topographic features of such landscapes is key (Waddington and Roulet, 1996).

Soils are capable of both producing and consuming CH_4 through the respective activity of methanogenic and methanotrophic microbial communities (Conrad, 1996). Production or methanogenesis results from the activity of obligate anaerobic methanogenic archea and syntrophic, hydrolytic, fermentative and acetogenic bacteria (Drake et al., 2009). In the majority of soil environments, methanogenesis occurs through hydrogenotrophic or acetotrophic pathways; respectively, the reduction of carbon dioxide (CO_2), using hydrogen as an electron donor, to CH_4 and the cleaving of acetate, reducing methyl-groups to CH_4 and oxidising carboxyl-groups to CO_2, by fermentation (Zinder, 1993; Conrad, 1999; Le Mer and Roger, 2001). These reactions are energetically unfavourable sinks for hydrogen and acetate in comparison to metabolic reactions utilised by other microbial communities common to soil environments. In this sense, methanogenesis represents the terminal step in carbon (C) mineralisation once electron acceptors, such as oxygen (O_2), nitrate, ferric iron and sulphate, involved in more energetically favourable metabolisms exploiting these substrates are depleted (Achtnich et al., 1995;

Chidthaisong and Conrad, 2000). In contrast, consumption or methanotrophy results from the oxidation of CH_4 to CO_2, using O_2 as an electron acceptor, by aerobic methanotrophic bacteria. In oxic environments enriched with CH_4, such as those with in situ methanogenesis, methanotrophy is associated with the activity of low-affinity communities whilst high-affinity groups dominate, most notably in environments at ambient atmospheric concentrations, where availability is low (Bender and Conrad, 1992; Hanson and Hanson, 1996; Knief et al., 2006). In this context, methanogenesis and methanotrophy have contrasting ecological requirements, principally, related to constraints imposed by O_2. The presence of O_2 is toxic to methanogenic archaea and precludes the conditions required for methanogenic metabolic pathways to be an energetically competitive sink for substrates, whereas, methanotrophic bacteria are micro-aerophilic requiring O_2 concentrations in the percent range (Bender and Conrad, 1994; Teh et al., 2006). The distribution of O_2 within soils is principally influenced by interaction between the structure of soil pore networks, water contents through slower liquid relative to gas-phase rates of diffusion and O_2 demand of aerobic respiration. For this reason such communities inhabit different ecological niches within the soil matrix and as such are likely to experience disparate stresses in response to variations in environmental conditions which in turn drive variations in soil-atmosphere CH_4 exchange.

Sources of CH_4 to the atmosphere are typically associated with inundated environments where the water table imposes a vertical stratification on metabolic activity (Conrad, 1996). In the saturated zone, O_2 is depleted as aerobic respiration outstrips recharge through downward diffusion across the air-water interface. Subsequently alternative electron acceptors are successively depleted under anaerobic conditions culminating in methanogenesis at depth. Indeed, wetland soils represent the largest natural source of CH_4 to the atmosphere accounting for 20 to 40 $\%$ of the annual global source budget (Denman et al., 2007). Methanotrophy can also be of importance in these environments, with low-affinity communities exploiting the abundance of both CH_4 and O_2 in superficial oxic layers, acting to consume significant amounts of endogenous CH_4 in situ (Frenzel and Karofeld, 2000; Hornibrook et al., 2009). Plants may play an important role in this respect by facilitating the transport of CH_4 into the soil profile through root networks (King, 1994; Calhoun and King, 1997; Fritz et al., 2011). In

such environments, variations in water table depth and temperature play central roles in determining net CH_4 fluxes reflecting variations in the balance between anoxic and oxic environments and temperature sensitivity of metabolic processes (Turetsky et al., 2014). Alternatively, sinks for atmospheric CH_4 are usually associated with unsaturated, upland soils where limited potential for flooding allows relatively free diffusion of air into soil pores. Such environments are thought to account for ~ 6 $\%$ of the annual global CH_4 sink budget (Dutaur and Verchot, 2007; Denman et al., 2007). Uptake of CH_4 in such soils occurs through the activity of high-affinity methanotrophs operating at close to ambient CH_4 and O_2 concentrations (Bender and Conrad, 1992). Under such conditions, oxidation is unsaturated with respect to CH_4 and variations in soil-atmosphere CH_4 exchange arise from constraints on the rate of CH_4 diffusion imposed by soil texture and water content (Bender and Conrad, 1992; Smith et al., 2003; Reay and Nedwell, 2004; Teh et al., 2006). Despite predominantly oxic conditions, upland soils have been shown to support anaerobic microbial processes within anoxic microsites (Sexstone et al., 1985; Peters and Conrad, 1996; Megonigal and Guenther, 2008). These zones of low O_2 concentration form through limitations on diffusion into soil pores occluded by aggregates and saturation combined with in situ O_2 uptake through respiration by roots and aerobic heterotrophs (Verchot et al., 2000; Teh et al., 2005, 2006). In 'transitional' or wet, fine textured soils with high rates of oxic respiration anaerobic processes may be considerable and potentially lead to emissions of CH_4 (Silver et al., 1999; Verchot et al., 2000; Teh et al., 2005). Similarly to wetland settings, soil-atmosphere CH_4 exchange is influenced by the balance between anaerobic and aerobic environments with low-affinity methanotrophic communities utilising elevated CH_4 concentrations close to the interface between anoxic and oxic niches (Conrad, 1996; Teh et al., 2005). For these reasons, soil-atmosphere CH_4 exchange in upland soils is typically related to water-filled pore space (WFPS) as an integrated proxy for diffusional limitations on the supply of atmospheric CH_4 to high-affinity methanotrophic communities and the distribution of O_2 controlling the balance between gross methanogenesis and methanotrophy (Verchot et al., 2000; Megonigal and Guenther, 2008; Von Fischer et al., 2009). In transitional soils, Verchot et al. (2000) further indicated the importance of soil CO_2 flux, reflecting biological O_2 demand, in

soils with high WFPS for promoting CH_4 emissions. Despite this, field studies relating soil CH_4 cycling and O_2 concentration are limited with the bulk of direct support for this mechanism focused on Puerto Rican montane tropical forests (Silver et al., 1999; Teh et al., 2005; Liptzin et al., 2011). In these respects, the general response of soil CH_4 cycles to variations in environmental conditions reflects the predominance and distribution of activity facilitating production and the consumption of both atmospheric and endogenous CH_4.

In predominantly oxic soils with minimal development of anoxic microsites soil-atmosphere CH_4 exchange varies in response to constraints on the supply of CH_4 to communities of high-affinity methanotrophy. Whereas in wetland and transitional soils CH_4 exchange, modulated by transport processes, is the product of the balance between methanogenic and methanotrophic activity. In such environments methanotrophic activity, particularly in settings with thick oxic layers surrounding zones of production, may determine soil-atmosphere exchange by consuming most or all of the endogenously produced CH_4. Indeed, if this low-affinity methanotrophy is sufficient soils may act to uptake atmospheric CH_4 through the activity of high affinity methanotrophs despite production at depth (Von Fischer and Hedin, 2007). However, the function of such soils as source or sink for atmospheric CH_4 appears to be ultimately determined by the ability of methanogenic communities to exploit anoxic conditions as methanotrophic activity is less sensitive to temperature than soil C mineralisation associated with methanogenesis and dependant on the supply of CH_4 (Segers, 1998; Teh et al., 2005; Von Fischer and Hedin, 2007). In this respect, controls on the supply of substrates to methanogens related to zones of readily available labile C associated with fresh litter and root exudates (Whiting and Chanton, 1993; Dannenberg and Conrad, 1999; Wachinger et al., 2000; Von Fischer et al., 2010) and competition with more energetically favourable metabolisms (Von Fischer and Hedin, 2007; Teh et al., 2008a; Hall et al., 2013) may represent important constraints on soil CH_4 cycling.

Here we report net CH_4 fluxes and associated environmental conditions from a humid puna grassland-wetland complex in southeastern Peru. We have previously reported the first year of this data in Teh et al. (2014) and suggest that the study site in a significant

source of CH_4 to the atmosphere, associated with anoxia within topographic lows, when compared to sink activity in the surrounding montane forests. These preliminary data indicated that temporal variations in soil-atmosphere CH_4 exchange principally occurred between rather than within seasons. Equally, considerable spatial variation was identified both between and within the topographic features considered. Here we report data from long-term measurements from January 2011 through June 2013 and seasonal intensive measurement campaigns from the 12^{th} through 22^{nd} of November 2011 and 12^{th} through 21^{st} of August 2012. Using long-term observations we aim to 1) test the source activity and seasonality of CH_4 emissions indicated by Teh et al. (2014) through analysis of a further 18 months of observations and 2) provide context to more detailed understanding of CH_4 cycling within these soils from the intensive seasonal campaigns. Using the intensive seasonal campaigns observations we aim to 3) characterise edaphic conditions for meso and microscale topographic variations across the study site, 4) investigate spatial controls on soil-atmosphere CH_4 exchange across and within landscape features in terms of edaphic and environmental conditions and 5) investigate the relationship between gross methanotrophic and methanogenic process rates in these soils. In these contexts, we hypothesise that variations in net CH_4 flux will be best explained by soil O_2 concentration as a integrated measure of anoxia across upland and wetland soil settings and that methanogenic processes will be active in the upland soils of this landscape despite oxic conditions.

4.3 Materials and methods

4.3.1 Study site

The study was carried out at Tres Cruces ($13°07'19''$ S, $71°36'54''$ W) on the western border of Manu National Park in the southeastern Peruvian department of Cusco. The area between ridge tops at ~ 4000 m asl and the treeline at ~ 3400 m asl has previously been described as humid puna grassland and broadly falls along the watershed dividing western xeric puna highlands and eastern steeply sloped evergreen tropical montane cloud forests bounding the Amazonian basin (Zimmermann et al., 2010b;

Gibbon et al., 2010). Mean annual precipitation is 1900 to 2500 mm and mean annual air temperature, at 3600 m asl, is 11 $^{\circ}\mathrm{C}$ (Gibbon et al., 2010). Precipitation is intensely episodic and has a pronounced wet season between October and April (Figure 4.1). In contrast, diurnal differences in air temperature are greater than seasonal variations. This is particularly notably during the dry season where decreases in minimal air temperature associated with cloud free nights commonly result in morning frosts.

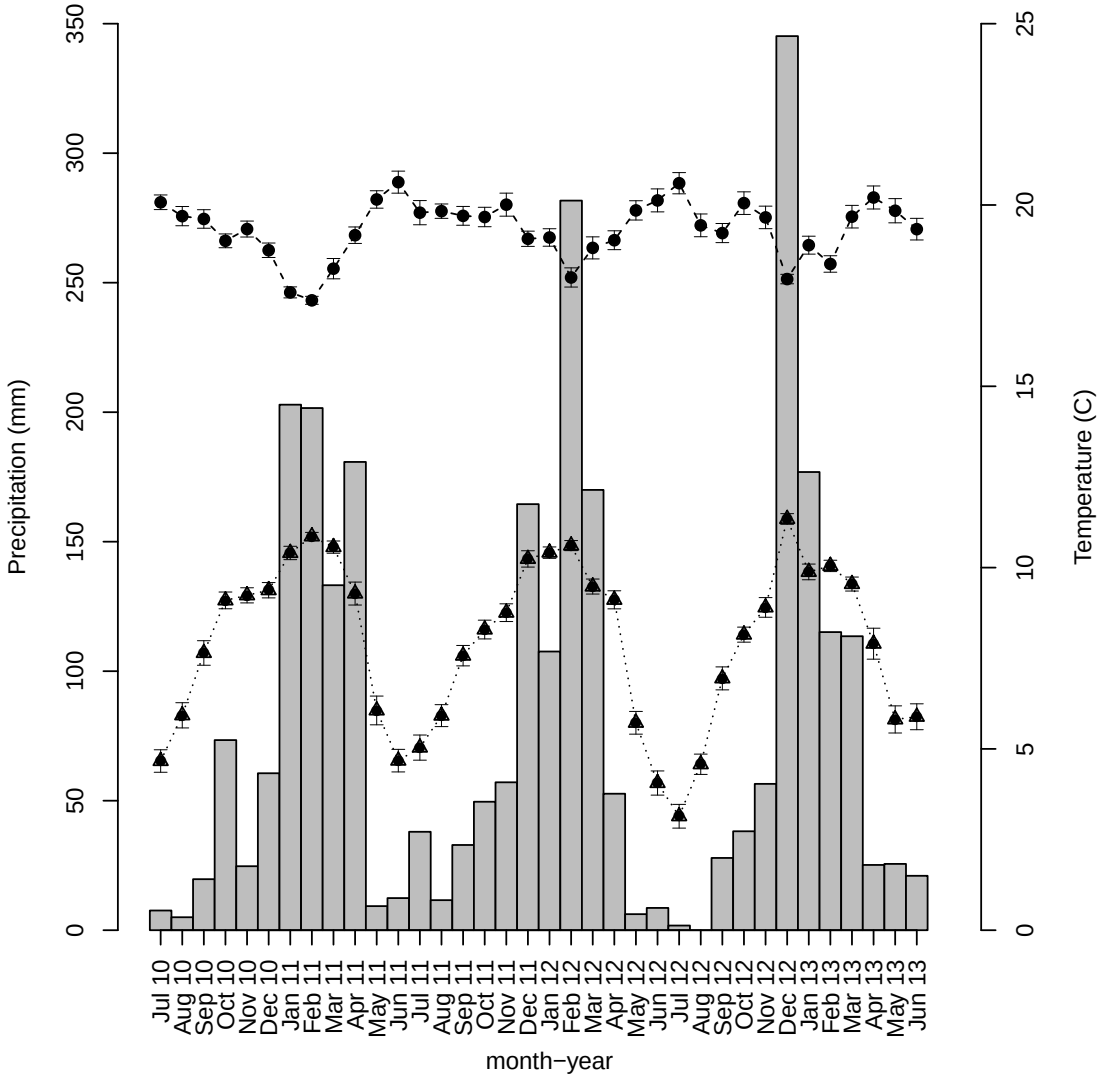

Figure 4.1: Total monthly precipitation and monthly mean maximum (•) and minimum (▲) diurnal air temperatures between July 2010 and June 2013 at 2808 m asl (Challabamba weather station: $13^{\circ}13'03''$ S $71^{\circ}38'50''$ W). Temperature error bars are standard error.

The puna at this site is characterised by upland ridge and slopes dominated by tussock grasses and basins containing wetland environments up to 1 ha in area (Figure 4.2). These wetlands consist of peat forming depressions, moss filled hollows and shallow

lakes of varying permanency (Figure 4.3). The study site has a history of cattle grazing by local communities and the environment at the landscape scale is susceptible to burning during dry periods (Gibbon et al., 2010; Rehm and Feeley, 2013; Oliveras et al., 2014a). Typical of paramo and wetter puna ecosystems, above-ground biomass is dominated by tussocks of *Calamagrostis* spp. and lower abundances of other grasses such as *Scirpus* spp., *Festuca* spp. and *Juncus* spp., in addition to mosses in moist locations and diverse herbs, shrubs and ferns (Luteyn and Churchill, 1999; Gibbon et al., 2010; Oliveras et al., 2014b). On upland ridges and slopes, soils are 20 to 40 cm in depth, have no O horizon and consist of a thick organic rich A horizon overlying a thinner stony B/C horizon. The surficial organic-rich mineral soils are acidic and typically have bulk densities on the order of ~ 0.40 $\mathrm{g\,cm^{-2}}$, C contents of ~ 15 $\%$ C and carbon to nitrogen (C:N) ratios of ~ 14 (Zimmermann et al., 2010b). Wetland peat soils range from 40 to over 100 cm in depth and, typically of such soils, have low bulk densities and C contents in excess of 40 $\%$ C (Zimmermann et al., 2010b). These peats are well humidified in basin settings where a mixture of moss and grass species occur but may develop extensive accumulations of moss litter in persistently wet hollows. The composition of the sub-soil in this environment reflects the Palaeozoic shale-slate geology of the region (Girardin et al., 2010).

Soil-atmosphere CH_4 exchange for 2011 has previously been reported for this site, as part of a study investigating non-CO_2 trace gas fluxes along an Andean altitude transect, by Teh et al. (2014). These measurements indicate that the puna at this location is a considerable source of CH_4 to the atmosphere, with a mean of 15.60 (2.14) mg CH_4-C m^{-2} d^{-1}, when compared with uptake activity dominant in soil-atmosphere exchange in the surrounding montane forests. These emissions were largely driven by basin features with high variability in weak source and sink activity from slopes and ridges. Seasonal patterns were identified with greater fluxes during the wet season than dry season but no within season trends were identified between months or on daily timescales during the wet season intensive campaign. Greater fluxes from the puna than the surrounding forests was attributed to near saturated soils and subsequent lower soil O_2 concentrations.

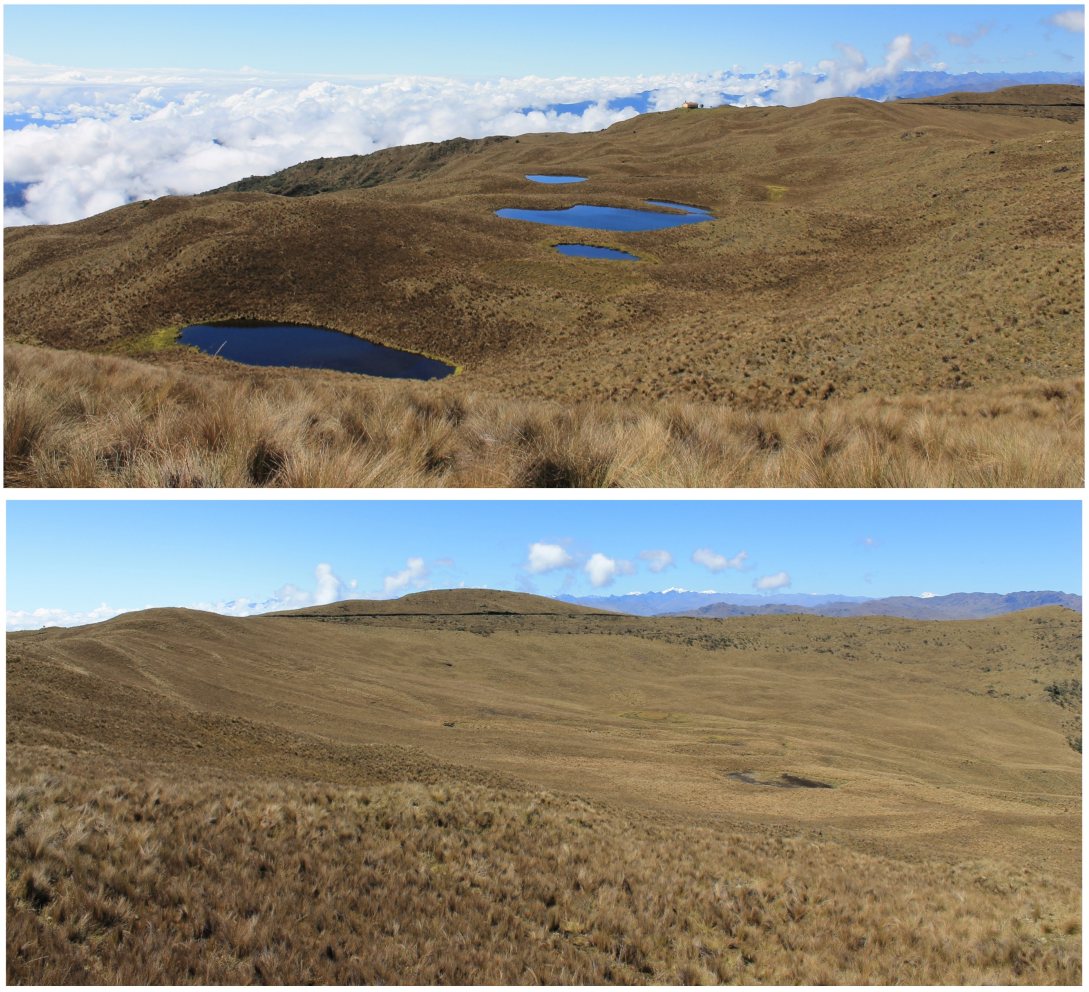

Figure 4.2: Typical landscapes at Tres Cruces: ridges and slopes covered by tussock grasses and basins containing topographically constrained wetland features. The lower image depicts the ridge to basin transition referred to in this study.

Figure 4.3: A variety of wetland features are identified within the study area: A) lakes of varying degrees of permanency, B) depressions containing well humidified peat soils and pool complexes, C) Hollows dominated by significant accumulations of mosses and their litter.

4.3.2 Sampling approach

As previously described in (Teh et al., 2014), this study focused on a northeast facing ridge to basin transition at ~ 3650 m asl. The transition from a narrow ridge, through of slopes of ~ -15° to the beginning of the basin occurs over ~ 200 m and ~ 50 m decrease in elevation. A broad basin extends northeast from the foot of the slope for a further ~ 100 m with little change in elevation before terminating in an escarpment where slope angles increase once again and eventually lead into the forests occupying the steeply inclined valleys that drain this landscape. To investigate the controls on soil-atmosphere CH_4 exchange from this environment measurements were taken at two temporal and spatial scales (Figure 4.4). In both cases sampling equipment was installed a minimum of 4 weeks prior to measurements to minimise the influence of disturbances and visted between the hours of 08:00 and 16:00 to minimise effects of temporal variability associated with sunrise and sunset at ~ 06:00 and 18:00 (Varner et al., 2003).

To investigate seasonal variability in soil-atmosphere CH_4 exchange four plots were established for long-term measurement campaigns on dominant landscape features encompassing the upper and lower reaches of the study area; specifically, a plot on the ridge and slope and in the basin a plot in a depression and a hollow. Each plot was instrumented at five sampling stations ~ 5 to 10 m apart. In addition to soil collars inserted at each sampling station, soil gas equilibration chambers were buried at three stations per plot. At each sampling station, soil-atmosphere gas exchange, soil moisture, soil temperature and, where soil-gas equilibration chambers were present, soil O_2 concentration were measured monthly. Measurements at the ridge, slope and depression plots were carried out, inclusively, from January 2011 to June 2013. The hollow plot was established at a later date than these plots with measurements beginning in August 2011. Measurements were not possible in July and December 2012 and February 2013 due to access restrictions.

To investigate spatial variability in soil-atmosphere CH_4 exchange and soil CH_4 cycling, intensive measurement campaigns were carried out in both the wet and dry sea-

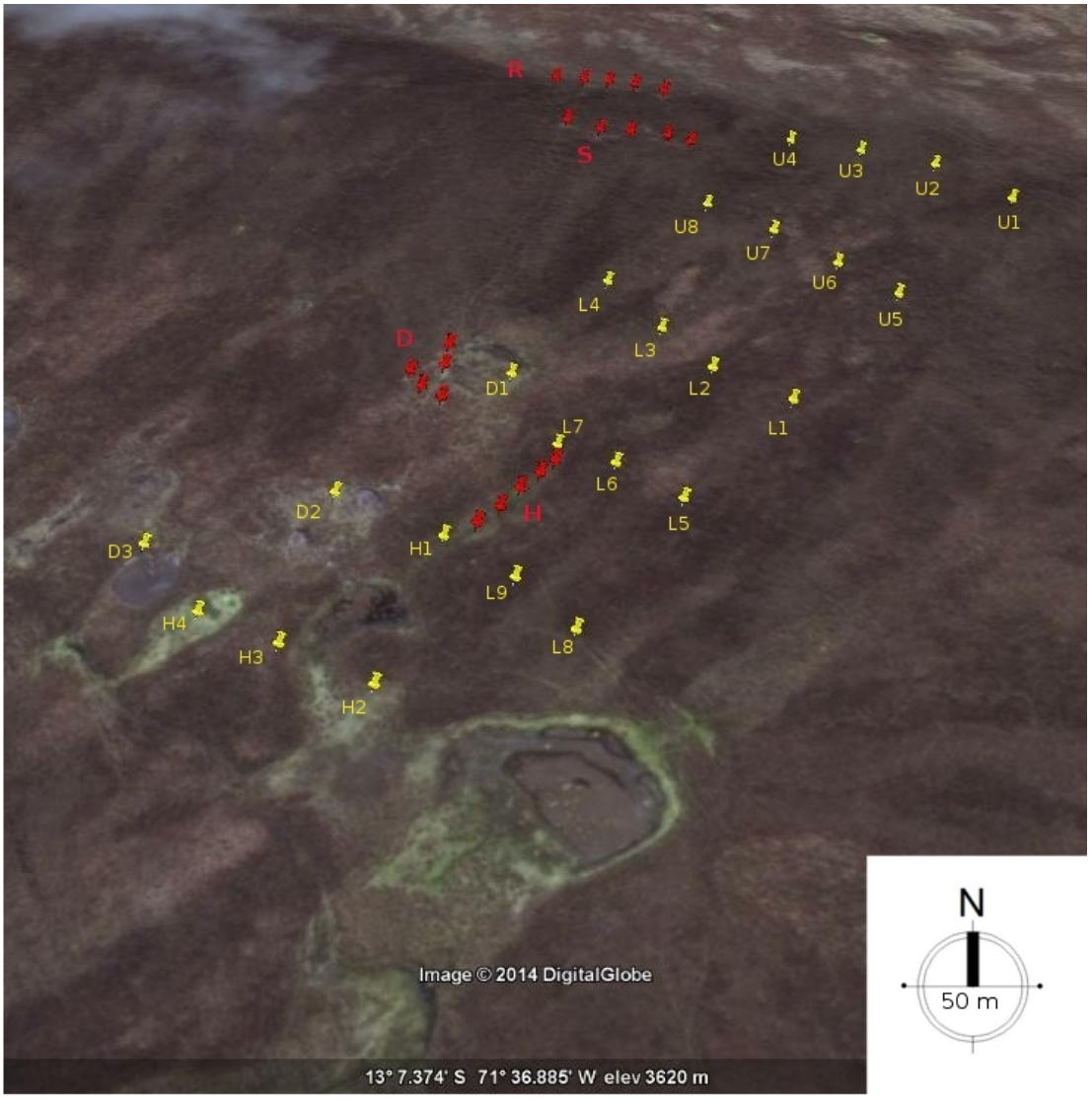

Figure 4.4: Distribution of sampling stations within the study area. Red pins indicate long-term measurement plots on ridge (R), slope (S), depression (D) and hollow (H). Yellow pins indicate sampling stations for the intensive measurement campaigns with upper slopes (U), lower slopes (L), depressions (D) and hollows (H) indicated (image: Google Earth).

son. A stratified sampling grid consisting of 24 sampling stations encompassing the topographic transition was established along six ~ 75 m transects running perpendicular to the principle northeast-southwest trend in slope. Each transect consisted of four sampling stations with a footprint of ~ 0.5 m^2 positioned ~ 25 m apart. The transects had a down-slope separation of ~ 50 m. Prior to each campaign, running from the 12^{th} to 22^{nd} of November 2011 in the wet and 12^{th} to 21^{st} of August 2012 in the dry season, each sampling station was equipped with a soil collar, soil gas equilibration chamber and a dip well. Sampling stations were visited daily to measure soil-atmosphere gas exchange, soil moisture, soil temperature, water table depth and soil O_2 concentration. On the last day of each campaign soil gas equilibration chambers were also sampled to determine soil trace gas concentrations. The equipment was removed following the wet season campaign and replaced directly adjacent to its previous position on undisturbed ground prior to the dry season campaign. Following the wet season campaign, the footprints of each soil collar were sampled to provide material for incubation experiments to investigate gross process rates and to characterise soil properties. Following the dry season campaign a vegetation survey was conducted at each sampling station to characterise vegetative community composition and above ground biomass .

4.3.3 Field measurements

4.3.3.1 Soil-atmosphere gas exchange

Soil-atmosphere gas exchange, focusing on fluxes of CH_4 and CO_2, was determined monthly in the long-term plots and daily during the intensive seasonal campaigns using a static chamber approach (Livingston and Hutchinston, 1995). Measurements were initiated by gently sealing, with a section of inner tube, cylindrical caps to pre-inserted 20 cm diameter soil collars to create a chamber of ~ 0.08 m^3 over a soil surface of ~ 0.03 m^3. Collars were inserted to a depth of ~ 5 cm. Caps were equipped with a gas sampling port, air pressure equilibration port, and a 9 V computer fan (Pumpanen et al., 2004). Ambient air temperature at 5 cm above the surface, chamber air temperature and atmospheric pressure were measured and a 20 ml gas sample taken at four

discrete time steps over a period of ~ 35 min following the initiation of a measurement. Gases were sampled using a stopcock and 60 ml gas tight syringe and were stored in an over-pressured, pre-evacuated 12 ml Exetainers (Labco Ltd., UK). Temperatures and atmospheric pressure were measured using a type K thermocouple (Omega Engineering Ltd.,UK.) and a Garmin GPSmap 60CSx (Garmin Ltd.,USA). Subsequently, CH_4 and CO_2 concentrations were determined by gas chromatography.

Fluxes, in $\mu\mathrm{l\,l^{-1}\,m^{-2}\,s^{-1}}$, were calculated in R (R Core Team, 2013) using the HMR package (Pedersen, 2012). Following the criteria outlined by Pedersen et al. (2010), HMR or linear models were fitted to time series of concentration in chamber headspaces. Significance was determined as $p < 0.05$ with emission and uptake indicated by positive and negative flux values, respectively. Fluxes were converted from concentration to amount basis (i.e. mg CH_4-C m^{-2} d^{-1} and g CO_2-C m^{-2} d^{-1}), following the ideal gas law, using a measurement average of chamber temperature and atmospheric pressure. A detection limit for each flux is calculated from the regression coefficients estimated in Parkin et al. (2012) appropriate to the model fitted at a measurement precision for CH_4 and CO_2 amount, respectively, 2.6 and 1.3 $\%$ (CV for air, $n > 30$). Significant fluxes below detection limits were reported to minimise bias (Gilbert, 1987). Non significant fluxes below detection limits were deemed to be net zero fluxes (i.e. reported as indeterminable or zero fluxes), whilst those greater than detection limits were considered to have resulted from failures during sampling, storage or analysis (i.e. reported as failures or 'NA'). In both cases these values were excluded from statistical analysis.

4.3.3.2 Soil gas concentrations

Soil O_2 concentration was measured monthly at three sampling stations per plot in the long-term plots and daily at every sampling station during the intensive seasonal campaigns. Additionally, on the final day of each intensive campaign samples were collected and stored to determine soil concentrations of CH_4 and CO_2. Soil gas equilibration chambers were vertically buried and centered at 10 cm below the soil surface (Silver et al., 1999; Teh et al., 2005; Liptzin et al., 2011; Hall et al., 2013). Chambers had interval volumes of 50 ml and surface areas of 57 $\mathrm{cm^2}$. Each consisted of

a length of gas-permeable silicone rubber tubing (AP202/60 - 35 mm inner diameter x 1.5 mm wall, Advanced Polymers Ltd, UK) sealed at one end with silicone cement and the other with a butyl rubber bung. A suitable length of tygon tubing was passed through a hole in the bung and capped with a stopcock to allow sampling at the surface. Chambers were encased in plastic mesh to protect the membrane during installation. Chambers were installed by coring a 3.5 cm diameter hole to the required soil depth, inserting the chamber and then back filling with the removed soil so that the stopcock emerged at the surface. Typical of similar designs, soil gas equilibration chambers were capable of equilibrating with the external atmosphere in less than 24 h (Holter, 1990; Jacinthe and Dick, 1996; Kammann et al., 2001).

Measurements of soil O_2 concentration were made by withdrawing 40 ml of gas from a soil-gas equilibration chamber using a stopcock and gas tight syringe. The sample was then passed through the flow-through head of an MO-200 oxygen sensor (Apogee Instruments Inc., USA) into a second syringe. The O_2 concentration was recorded, with a precision of 0.1 $\%$, and the gas sample re-injected into the soil-gas equilibration chamber from the second syringe. Prior to measurements the O_2 sensor was calibrated, as required, in field with air and the dead volumes within the sampling apparatus evacuated to minimise contamination of the soil gas sample by residuals. In exception to this method, on the final day of the intensive campaigns rather than returning the sample to the soil-gas equilibration chamber the sample was stored in an over-pressured, pre-evacuated 12 ml Exetainer (Labco Ltd., UK) for determination of CH_4 and CO_2 concentration by gas chromatography.

4.3.3.3 Soil water content and temperature

Soil water content and soil temperature were measured in triplicate at each sampling station. During the long-term measurements, soil water content was determined at 12 cm below the surface using a ML2x ThetaProbe equipped with 12 cm rods (Delta-T Ltd., UK). During the intensive measurement campaigns, soil water content integrated over the upper 20 cm of soil was determined daily using a CS620 Hydrosense unit equipped with 20 cm rods (Campbell Scientific Inc, USA). For the intensive campaign

measurements WFPS was calculated from these data using percentage water content and an estimate of porosity for each sampling station. For the long term measurements volumetric water content (VWC) is reported as plot estimates of porosity are unavailable. Water table depth was also determined daily during the intensive measurement campaigns. Dip wells, constructed from 35 mm plastic tubing, were inserted to a depth of 20 cm. Water table depth was determined from the displacement of a float relative to the soil surface and reported to a maximum depth of 20 cm to account for variations in total soil depth across the study area. Soil temperature for both measurement scales was measured at 10 cm using a type K penetration probe (Omega Engineering Ltd.,UK).

4.3.4 Site characterisation

4.3.4.1 Soil sampling

Following the intensive wet season campaign, soil depth to the B/C horizon was measured and the soil sampled at each sampling station. Paired soil samples were taken from 0 - 5 and 5 - 15 cm within the soil collar footprint. To sample the surface soils between 0 - 5 cm, vegetation was clipped to the soil surface and samples consisting of a block 10 by 5 by 5 cm, with a volume of 250 cm^3, removed using scissors. Soils from 5 - 15 cm depth were sampled using a 10 cm long, 50 mm diameter corer to yield a sample with a volume of 173 cm^3. Soil depth was determined by inserting a metal rod until physical resistance to further insertion was encountered (Zimmermann et al., 2010b). One sample from each depth pair was processed by gently homogenizing and removing root fragments prior to use in incubations experiments and determination of soil properties. The second sample from each depth pair was kept intact to determine bulk density.

4.3.4.2 Vegetation survey

A vegetation survey was carried out in Septermber 2012 following the intensive dry season campaign. At each of the sampling locations two 0.25 m^2 quadrats were placed,

perpendicular to the slope, at either side of each sampling station. Additionally, slope angle was measured in the principle downslope, northeast-southwest, orientation and perpendicularly across slope using a clinometer and ranging poles. Within each quadrat every non-bryophyte was identified to the genus or species level. The biomass associated with dominant grass genera, *Calamagrostis* sp., *Scirpus* sp. and *Juncus* sp., was determined following the optimal allometric model developed for these grasslands by Oliveras et al. (2014b). Tussock basal and crown diameters was measured along the longest and perpendicular axes to calculate basal and crown areas (Johnson et al., 1988). Tussock height was measured when stretched by hand. Above ground biomass was calculated as,

$$ABG = a(BA)^b * (H^c) * (CA^d) \tag{4.1}$$

where, AGB is above ground biomass (g), BA is the basal area (cm^2), CA is crown area (cm^2), h is height (cm) and the genera specific coefficients, reported in Oliveras et al. (2014b), are a, b, c and d. The above ground biomass for each tussock was summed for each pair of quadrats to give total and genera specific above ground biomass density ($\mathrm{g\,m^{-2}}$) at each sampling location.

4.3.5 Laboratory-based ^{13}C isotope tracer study

Approximately 100 g of soil, at field water content, from both depths at each sampling station collected following the wet season intensive campaign, were placed in 1 l Kilner jars. Blanks consisted of jars containing no soil. Jars were loosely sealed with screw-cap lids fitted with septa ~ 48 h before the start of measurements. To initiate incubations, jars were vented and fully sealed before being spiked with 1 ml of nitrogen carrier (N_2) with sulfur hexafluoride (SF_6) and $^{13}CH_4$ concentrations of ~ 0.3 and 70 $\mu\mathrm{l\,l^{-1}}$, respectively. Jar headspace was mixed with a 60 ml syringe and pre-incubated for 30 to 60 min to allow the tracers to fully equilibrate within the headspace (Von Fischer and Hedin, 2002). Following the pre-incubation period, 100 ml of N_2 was

injected and the headspace mixed at four discrete times over the course of 10 to 12 h. The resulting overpressure was sampled and stored in evacuated 60 ml Wheaton bottles sealed with 20 mm butyl septa (Geo-microbial Technologies Inc., USA). Subsamples of 5 ml were taken from each bottle for analysis by gas chromatography, with measurements corrected for sampling dilution, and the remainder analysed by isotope ratio mass spectrometry. Incubations took place in the dark at 24 $^{\circ}\mathrm{C}$. Incubations were leak checked based on changes in the concentration of SF_6. Headspace volume was calculated based on oven dry soil mass, gravimetric water content and particle density.

4.3.5.1 Isotope pool dilution model

The ^{13}C-tracer isotope pool dilution model developed and the assumptions described by Von Fischer and Hedin (2002) was used to deconvolve the contribution of gross consumption and production of CH_4 to the net change in amount of CH_4 in incubation headspace. Following Michaelis-Menten kinetics and with the assumptions that CH_4 concentration is below K_m (the CH_4 concentration at which oxidation is half the maximum rate for the system) (Bender and Conrad, 1992) and that production contributes negligible amounts of $^{13}CH_4$ over the course of the incubation period, the rate of consumption can be determined from the first order decay of the amount of $^{13}CH_4$ (Von Fischer and Hedin, 2002), described by,

$$[^{13}CH_4]_t = [^{13}CH_4]_0 \exp^{-k_{13}t} \tag{4.2}$$

where $[^{13}CH_4]_t$ is the concentration of $^{13}CH_4$ at a given time, $[^{13}CH_4]_0$ is the concentration of $^{13}CH_4$ at time zero, k_{13} is the first order decay rate for the consumption of $^{13}CH_4$ and t is time since the beginning of the incubation.

The balance between the rate of production and consumption is determined from relationships between amount of CH_4 and the atom percent of $^{13}CH_4$ against time and each other. When consumption is greater than production, the amount of CH_4 decreases with time and a positive relationship is observed between amount of CH_4 and atom

percent of $^{13}CH_4$ as $^{12}CH_4$ is preferentially consumed with respect to $^{13}CH_4$. When production is greater than consumption, the amount of CH_4 increases with time and a negative relationship is observed between amount of CH_4 and atom percent of $^{13}CH_4$ as the headspace is diluted by isotopically light CH_4. When process rates are equal the amount of CH_4 will remain constant with respect to time and the atom percent of $^{13}CH_4$ will decrease from time zero at a constant concentration of CH_4. The change in amount of CH_4 is described by,

$$[CH_4]_t = \frac{P}{k_{12}} - \left(\frac{P}{k_{12}} - [CH_4]_0\right)\exp^{-k_{12}t} \tag{4.3}$$

where $[CH_4]_t$ is the concentration of CH_4 in the system at a given time, P is the gross rate of CH_4 production, k_{12} is the first order decay rate for $^{12}CH_4$ and $[CH_4]_0$ is the concentration of CH_4 at time zero. The change in the ratio of the amount of $^{13}CH_4$ to CH_4 follows as,

$$\frac{[^{13}CH_4]_t}{[CH_4]_t} = \frac{[^{13}CH_4]_0 \exp^{-k_{13}t}}{\frac{P}{k_{12}} - \left(\frac{P}{k_{12}} - [CH_4]_0\right)\exp^{-k_{12}t}} \tag{4.4}$$

and,

$$AP_t = \frac{[^{13}CH_4]_t}{[CH_4]_t} + AP_p \tag{4.5}$$

where AP_t is the atom percent of $^{13}CH_4$ at a given time and AP_p is the atom percent of produced CH_4.

4.3.5.2 Determination of gross process rates

The rate of CH_4 consumption is calculated as,

$$C = bvk_{12} \tag{4.6}$$

where C is the rate of CH_4 consumption, b is the concentration of CH_4 at the soil surface and v is the volume of the headspace. A value of 1.8 $\mu l\, l^{-1}$ is used for b to standardise rates of C to atmospheric CH_4 concentration (Von Fischer and Hedin, 2002). k_{13} is estimated from the linear regression of the natural log of the concentration of $^{13}CH_4$ against time (equation 4.2) and k_{12} is then calculated as,

$$k_{12} = k_{13}\frac{1}{\alpha} \tag{4.7}$$

where α is the fractionation factor for methane consumption. A value of 0.98 was adopted for α with conceivable variations unlikely to introduce large biases (Von Fischer and Hedin, 2002).

The rate of CH_4 production, P, is estimated by simultaneously fitting equations 4.3 and 4.4 to observations of $^{13}CH_4$ and CH_4 concentration. P is calculated recursively to optimise the best solution via minimisation of the normalised total error between observed and predicted values. Normalised total error, E, is calculated as,

$$E = \left(\sum_{t=1}^{n} \frac{|AP_{obs}(t) - AP_{pred}(t)|}{SD_{AP_{obs}}}\right) N_{AP} + \left(\sum_{t=1}^{n} \frac{|[CH_4]_{obs}(t) - [CH_4]_{pred}(t)|}{SD_{[CH_4]_{obs}}}\right) N_{[CH_4]} \tag{4.8}$$

With normalisation factors for isotope data, N_{AP}, and concentration data $N_{[CH_4]}$ calculated as,

$$N_{AP} = \frac{SD_{AP_{obs}}}{SD_{AP_{prec}}} \tag{4.9}$$

and,

$$N_{[CH_4]} = \frac{SD_{[CH_4]_{obs}}}{SD_{[CH_4]_{prec}}} \tag{4.10}$$

where $SD_{AP_{obs}}$ and $SD_{[CH_4]_{obs}}$ are the standard deviations of observations and $SD_{AP_{prec}}$ and $SD_{[CH_4]_{prec}}$ is the analytical precision for mass spectrometry and gas chromatography measurements. To account for random variations associated with the experimental set-up, errors were minimised relative to variability observed in blank incubations where $SD_{AP_{obs}}$ and $SD_{[CH_4]_{obs}}$ were 0.01 and 0.48, respectively.

Recursive optimization was implemented using the BB package (Varadhan and Gilbert, 2014) in R (R Core Team, 2013) with a starting condition for P of 0 $\mu l\, l^{-1}\, s^{-1}$. The modelled net CH_4 flux rate, F, is calculated as the difference between P and C,

$$F = P - C \tag{4.11}$$

4.3.5.3 Total soil C mineralisation and the methanogenic fraction

To investigate the relationship between gross production of CH_4 and the availability of substrates, the rate of total soil C mineralisation and the methanogenic fraction of soil C mineralisation were estimated following Von Fischer and Hedin (2007). The methanogenic fraction of soil C mineralisation is,

$$MF = \frac{(R_{CH_4} + R_{CO_2})_{methanogenic}}{(R_{CH_4} + R_{CO_2})_{total}} \tag{4.12}$$

where MF is the methanogenic fraction of total soil C mineralisation and $(R_{CH_4} + R_{CO_2})$ are the rates of soil C converted to CH_4 and CO_2 by methanogenic pathways and the rate of total soil C mineralisation by both methanogenic and non-methanogenic pathways. Assuming that methanogenesis proceeds via fermentation and that CH_4 and CO_2 are produced in a ratio of 1:1 (Grant, 1998; Von Fischer and Hedin, 2007), total C mineralisation and the methanogenic fraction are calculated as,

$$MF = \frac{2(P_{CH_4})}{(P_{CH_4} + F_{CO_2})} \tag{4.13}$$

where P_{CH_4} is the modelled rate of CH_4 production and F_{CO_2} is the observed rate of CO_2 production.

4.3.6 Laboratory analyses

4.3.6.1 Gas chromatography

Gas samples collected during static chamber flux measurements, soil gas profile sampling and laboratory incubations were analysed by gas chromatography using a Thermo TRACE GC Ultra (Thermo Fisher Scientific Inc., USA) with a N_2 carrier gas. A flame ionization detector, methanizer-flame ionization detector and electron capture device were used to determine CH_4, CO_2 and SF_6 concentrations, respectively. Analytes were separated using a Hayesep Q 100/200 column. The gas chromatograph was equipped with a 2 ml sample loop and oven temperature was 60 $^{\circ}\mathrm{C}$. Detector responses were calibrated using three or more, triplicated, certified gas standards (CK Gas Products Ltd., UK) and instrumental precision was deemed acceptable when coefficient of variances < 5 $\%$ were achieved. A custom-built autosampler (University of York, UK) was used to introduce gas samples collected from static chamber flux measurements to the sample loop. Gas samples collected from soil gas equilibration chambers and laboratory incubations were manually injected into the sample loop using a 10 ml, low dead volume gas-tight syringe (VICI Precision Sampling, USA).

4.3.6.2 Isotope ratio mass spectrometry

The isotopic composition of C-CH_4 in incubation gas samples was analysed by isotope ratio mass spectrometry on a Finnigan Deltaplus XP GC-IRMS coupled to a Gasbench II and an automated trace gas PreCon (Thermo Fisher Scientific Inc., USA) at University of St Andrews, UK. Sample bottles were flushed into the PreCon over a period of 700 s by a stream of helium flowing at rate of 0.4 $\mathrm{ml\,s^{-1}}$. The gas stream passes through a chemical trap, containing magnesium perchlorate to remove water vapour and Carbosorb to remove CO_2, into a liquid nitrogen cryotrap which removes residual condensible gases. Non-condensible gases then pass into an oven, at 950 $^{\circ}\mathrm{C}$ with a nickel-

platinum catalyst, where CH_4 is oxidised to CO_2 and subsequently pre-concentrated in a second liquid nitrogen trap over a period of 320 s. CO_2 is then cryofocused in a third trap and injected into the GC-IRMS via the Gasbench. System linearity and precision across the sample concentration range was confirmed with an in-house methane standard with a $\delta\,^{13}$C value of -48.2 ‰ relative to VPBD and a coefficient of variance over the analysis period of 0.06.

4.3.6.3 Soil physical properties

The paired soil samples designated for the determination of bulk density were dried for 24 h at 105 $^{\circ}\mathrm{C}$ to determine oven dry mass. Bulk density was calculated as the oven dry mass per soil field volume of this sample. Subsequently, particle density was determined following the method described in Klute et al. (1986) and using 10 ml pycnometers. Porosity was estimated from these data as the difference between 1 and the fraction bulk and particle density.

4.3.6.4 Soil chemical properties

Soil pH was determined in a gravimetric 1:2 slurry of air dried soil and deionised water following Sparks et al. (1996) using a HANNA pHep 4 tester with a precision of 0.1 pH (HANNA Instruments, USA). Soil C and N content were determined using a Costech ECS4010 elemental analyser elemental analyser with a zero-blank autosampler (Costech Analytical Technology Inc., USA) coupled to a Finnigan Deltaplus XP GC-IRMS. Air dried soil was finely ground and 5 - 15 mg aliquots weighed out into tin capsules. Capsules were crimped, balled and weighed at a precision of 0.001 mg. Detector response was calibrated using triplicates of empty tin capsules as blanks and a certified soil standard, B2176, with 15.98 $\%$ C and 1.27 $\%$ N (Elemental Microanalysis Ltd., UK). Standards were prepared in the same manner as samples and at four content levels selected to encompass the range of C and N contents expressed in the soils as indicated during method optimisation. Coefficients of variance for standard C and N contents were 2 $\%$ and < 3 $\%$, respectively.

4.3.7 Statistical analysis

Statistical analyses were conducted in R version 3.1.1 with significance reported as $p < 0.05$ (R Core Team, 2013). Parametric or non-parametric statistical techniques were applied based on visual assessment of residuals (Zuur et al., 2007). A generalised least squares approach was used to reduce heteroscedasticity in investigating 1) the effect of season within long-term measurement plots on monthly means of net CH_4 and CO_2 fluxes, VWC, soil O_2 concentration and soil temperature, 2) the effect of microform type in intensive campaigns on net CH_4 and CO_2 fluxes, WFPS, water table depth, soil O_2 concentration and soil temperature, 3) the effect of microform type and depth on observed and modelled rates in the incubation experiment (Pinheiro et al., 2014; Zuur et al., 2009). The effect of microform type and sampling depth on soil chemical and physical properties were investigated through analysis of variance. Post-hoc multiple comparison of effects in these situations was conducted with Tukey contrasts using the multcomp package (Hothorn et al., 2008). Relationships between sampling station edaphic conditions were investigated through principal component analysis with scaled variables (Zuur et al., 2007). For the intensive campaigns, spatial relationships between edaphic conditions and the mean of wet and dry season campaign sampling station net CH_4 fluxes was investigated using Spearman's rank correlation coefficients due to considerable non-linearity and heteroscedasticity in these data. Similarly within seasonal campaigns, Spearman's rank was used to investigate relationships between campaign means of net CH_4 flux and WFPS, water-table depth, soil O_2 concentration, soil temperature, net CO_2 flux and soil gas concentrations. For the incubation experiments, linear regression was used to investigate relationships within sampling depth between observed and modelled rates.

4.4 Results: long-term measurements

4.4.1 Monthly variations in soil-atmosphere gas exchange and environmental conditions

Soil-atmosphere CH_4 and CO_2 exchange, soil O_2 concentration, VWC and soil temperature are reported monthly from ridge, slope and depression from January 2011 to June 2013 and from the hollow plot from August 2011 to June 2013. Means and standard errors for wet season months between October and April and dry season months between May and September are reported in Table 4.1. Net CH_4 fluxes were lower during dry season months than wet season months with means (standard errors) of -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} on the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} on the slope, -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} in the depression and 24.65 (10.7) and 181.74 (36.35) mg CH_4-C m^{-2} d^{-1} in the hollow, respectively. These seasonal differences were statistically significant for all plots with the exception of the depression. Within plot and month, spatial variability in net CH_4 flux was of a similar order of magnitude to between month variations (Figure 4.5). In this respect, wet season increases in net CH_4 fluxes were accompanied by notable increases in spatial variability within plots. No significant differences between wet and dry season months were observed in CO_2 fluxes with means of 1.01 (0.14) and 1.10 (0.13) g CO_2-C m^{-2} d^{-1} on the ridge, 1.26 (0.17) and 1.62 (0.29) g CO_2-C m^{-2} d^{-1} on the slope, 2.20 (0.41) and 2.16 (0.33) g CO_2-C m^{-2} d^{-1} in the depression and 1.90 (0.34) and 2.10 (0.22) g CO_2-C m^{-2} d^{-1} in the hollow, respectively. Similarly, there were no significant differences in VWC between wet and dry season months with means of 72.3 (1.0) and 72.7 (0.5) % on the ridge, 73.8 (0.6) and 73.9 (0.3) % on the slope, 72.7 (0.8) and 73.9 (0.5) % in the depression and 76.2 (0.2) and 75.6 (0.3) % in the hollow, respectively. Soil O_2 concentrations were lower during wet season than dry season months with means of 18.5 (0.6) and 16.4 (0.8) % on the ridge, 16.8 (1.0) and 13.1 (1.8) % on the slope, 16.1 (1.3) and 11.0 (1.4) % in the depression and 9.5 (3.3) and 5.3 (3.3) % in the hollow, respectively. These seasonal differences were statistically significant for the ridge and depression plots but not the slope or hollow plots.

Soil temperature was significantly greater during wet season than dry season months in all plots with means of 9.0 (0.3) and 11.5 (0.2) ℃ on the ridge, 8.6 (0.4) and 11.6 (0.3) ℃ on the slope, 9.3 (0.3) and 11.6 (0.3) ℃ in the depression and 9.1 (0.3) and 11.9 (0.3) ℃ in the hollow, respectively.

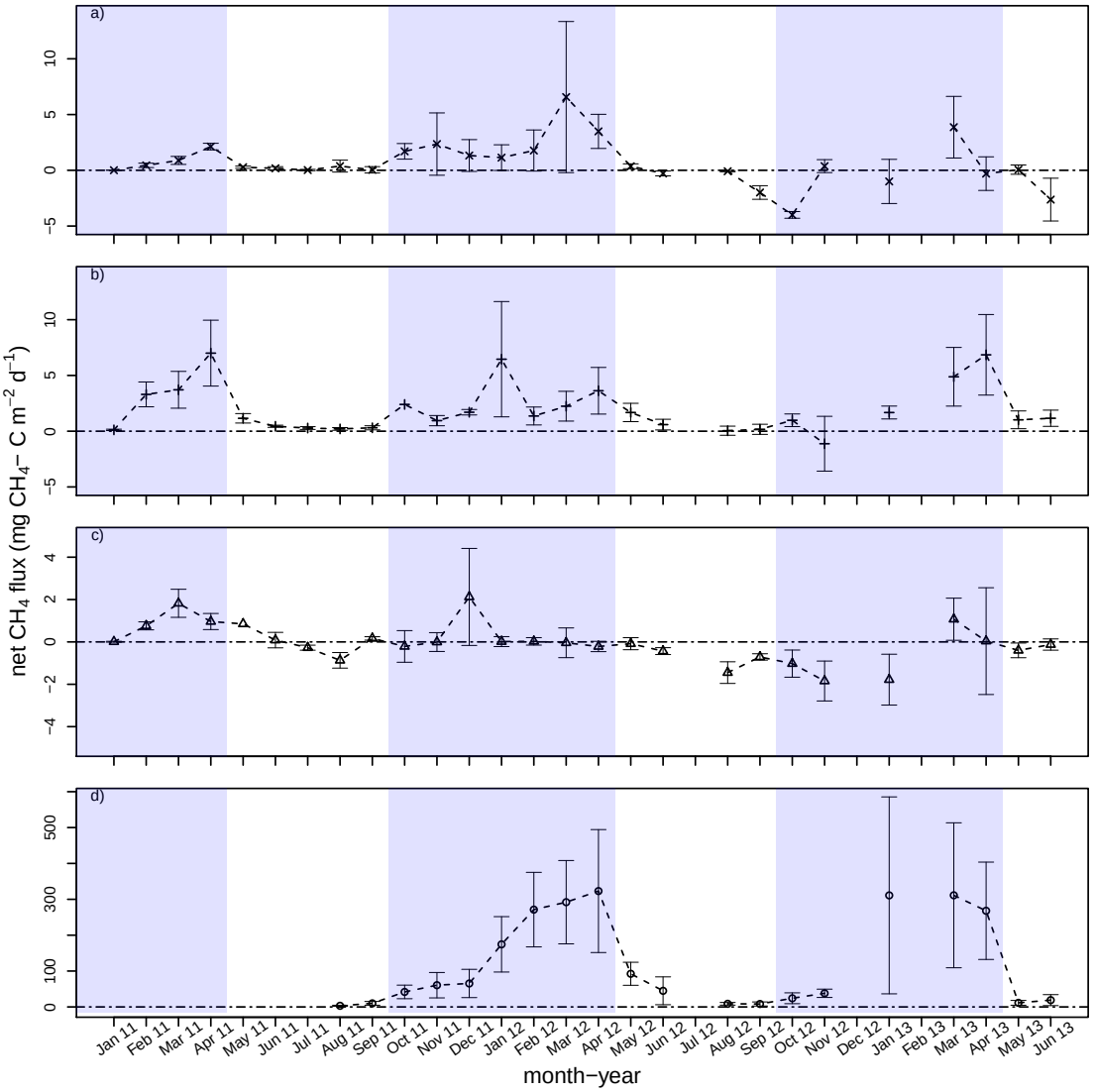

Figure 4.5: Monthly long-term measurement plot means of net CH_4 flux and standard error bars for a) ridge, b) slope, c) depression and d) hollow. Shading indicates wet season of October - April. n = 5 per plot for net CH_4 flux, net CO_2 flux, VWC and soil temperature and n = 3 per plot for O_2 concentration.

Table 4.1: Long-term measurement plot means (n = 5 per plot) and standard errors for aggregated dry (May - September; n = 11 for ridge, slope and depression and n = 8 for hollow) and wet (October - April; n = 16 for ridge, slope and depression and n = 12 for hollow) season months. Lower case letters indicate significant differences between season within plots.

	Plot	Ridge	Slope	Depression	Hollow
dry	Net CH_4 flux	-0.33 (0.30) a	0.64 (0.16) a	-0.30 (0.18) a	24.65 (10.70) a
wet	(mg CH_4-C m^{-2} d^{-1})	1.30 (0.58) b	2.88 (0.60) b	0.11 (0.27) a	181.74 (36.35) b
dry	Net CO_2 flux	1.01 (0.14) a	1.26 (0.17) a	2.20 (0.41) a	1.90 (0.34) a
wet	(g CO_2-C m^{-2} d^{-1})	1.10 (0.13) a	1.62 (0.29) a	2.16 (0.33) a	2.10 (0.22) a
dry	O_2 concentration	18.5 (0.6) a	16.8 (1.0) a	16.1 (1.3) a	9.5 (3.3) a
wet	(%)	16.4 (0.8) b	13.1 (1.8) a	11.0 (1.4) b	5.3 (3.3) a
dry	VWC	72.3 (1.0) a	73.8 (0.6) a	72.7 (0.8) a	76.2 (0.2) a
wet	(%)	72.7 (0.5) a	73.9 (0.3) a	73.9 (0.5) a	75.6 (0.3) a
dry	Soil temperature	9.0 (0.3) a	8.6 (0.4) a	9.3 (0.3) a	9.1 (0.3) a
wet	(°C)	11.5 (0.2) b	11.6 (0.3) b	11.6 (0.3) b	11.9 (0.3) b

4.5 Results: intensive seasonal campaigns

4.5.1 Edaphic conditions

4.5.1.1 Topography

Edaphic conditions at each of the twenty four sampling stations visited during the wet and dry season intensive campaigns were characterised. Sampling station elevation ranged from 3673 to 3629 m asl. Similarly, slope angles in the principal down slope, NE-SW, direction ranged from -19 to $0°$. Perpendicular to this, across slope angles range from -11 to $2°$ in the NW-SE direction. This transition from steep upper slopes to flat basin dominates environmental gradients within the study area.

Of the twenty four sampling stations, seventeen were situated on slopes and seven were located on flat or very gentle ground in the basin (Table 4.2). The sampling stations on the slopes were divided into two microform types, upper slopes and lower slopes, consisting of 8 and 9 locations respectively. Sampling stations on the upper slopes occurred at elevations of 3657 to 3673 m asl and on downslope angles of -19 to $-10°$. Similarly, sampling stations on the lower slopes occurred at elevations ranging from 3634 to 3649 m asl and on downslope angles of -12 to $-7°$. Within the basin two microform groups were identified, four sampling stations were located in moss-filled hollows whilst the remaining three stations were in depressions with complexes of peat soils and ephemeral pools. Elevations ranged from 3629 to 3637 m asl for the hollows and 3629 to 3639 m asl for the depressions. Slope angles ranged from -3 to $0°$ for both these basin features.

4.5.1.2 Vegetation

Twelve non-bryophyte genera; *Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp., *Hipericun andinun*, *Hypochaeris taxacoides*,*Viola pigmaea*, *Lysiponia lacinata*, *Halenia bella*, *Senecio bukartii*, *Vaccinium floribundum*, *Puya pigmmaea* and *Werneria nubigena*, were identified at the sampling stations. The grasses *Calamagrostis longiaris-*

Table 4.2: Edaphic conditions for microform groups identified in the intensive sampling campaigns. Numbers are reported as means and standard errors.[1] Down-slope angle, [2] Across slope angle and [3] Above ground biomass of *Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp. and the total of these.

Microform	Upper slope	Lower slope	Depression	Hollow
No. sampling locations	8	9	3	4
Elevation (m asl)	3663 (2)	3642 (2)	3634 (3)	3631 (2)
NE-SW angle[1] (°)	-14 (1)	-10 (1)	-2 (1)	-1 (1)
NW-SE angle[2] (°)	-2 (1)	-2 (1)	0 (0)	-1 (0)
Calamagrostis[3] ($\mathrm{g\,cm^{-2}}$)	272.9 (77.8)	105.6 (30.9)	119.0 (62.8)	0.0 (0.0)
Scirpus[3] ($\mathrm{g\,cm^{-2}}$)	10.7 (4.2)	25.0 (7.1)	11.1 (11.1)	13.2 (4.6)
Juncus[3] ($\mathrm{g\,cm^{-2}}$)	2.6 (2.4)	30.9 (11.3)	20.3 (17.0)	63.6 (5.6)
Total[3] ($\mathrm{g\,cm^{-2}}$)	286.2 (77.7)	161.4 (30.5)	150.4 (49.7)	76.8 (8.1)

tata, *Scirpus* sp. and *Juncus* sp. were most abundant with tussocks of the bunch-grass *Calamagrostis longiaristata* dominating the landscape. Above-ground biomasses associated with *Calamagrostis longiaristata*, *Scirpus* sp. and *Juncus* sp. respectively ranged from 0 to 659.9 $\mathrm{g\,m^{-2}}$, 0 to 61.6 $\mathrm{g\,m^{-2}}$ and 0 to 91.5 $\mathrm{g\,m^{-2}}$. Total above ground biomasses associated with these species ranged from 54.2 to 659.9 $\mathrm{g\,m^{-2}}$. The upper slope and hollow sampling stations were typified by high biomasses of *Calamagrostis longiaristata* and *Juncus* sp., respectively. Whilst there was no clear grouping of the lower slope and depression sampling stations with respect to these grasses.

Calamagrostis longiaristata, *Scirpus* sp., *Juncus sp.*, *Hypochaeris taxacoides*, *Halenia bella*, *Senecio bukartii*, *Vaccinium floribundum* and *Werneria nubigena* were identified at sampling stations on both the upper and lower slopes (Table 4.3). Additionally, *Hipericun andinun*, *Viola pigmaea*, *Lysiponia lacinata* and *Puya pigmmaea* were also present at upper slope sampling stations. Mosses were abundant in the basin sampling stations. In addition to these, the depression sampling stations contained *Calamagrostis longiaristata*, *Scirpus* sp., *Juncus sp.*, *Senecio bukartii*, and *Vaccinium floribundum*. Whilst, the hollows were notable for the absence of *Calamagrostis longiaristata* with only *Scirpus* sp. and *Juncus* sp. identified at these sampling stations. Above ground biomass of *Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp., and the total of these were, respectively; 272.9 (77.8), 10.7 (4.2), 2.6 (2.4) and 286.2 (77.7) $\mathrm{g\,m^{-2}}$ for upper slopes, 105.6 (30.9), 25.0 (7.1), 30.9 (11.3), and 161.4 (30.5) $\mathrm{g\,m^{-2}}$ for lower slopes, 119.04 (62.8), 11.1 (11.1), 20.3 (17.0) and 161.4 (30.5) $\mathrm{g\,m^{-2}}$ for depressions

and 0.0 (0.0), 13.2 (4.6) , 63.6 (5.6) and 76.8 (8.1) $\mathrm{g\,m^{-2}}$ for hollows (Table 4.2).

Table 4.3: Vegetation lists by microform group sampled during the intensive campaigns.

Microform	Vegetation list
Upper slope	*Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp., *Hypochaeris taxacoides*, *Halenia bella*, *Senecio bukartii*, *Vaccinium floribundum*, *Werneria nubigena*, *Hipericun andinum*, *Viola pigmaea*, *Lysiponia lacinata* and *Puya pigmmaea*
Lower slope	*Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp., *Hypochaeris taxacoides*, *Halenia bella*, *Senecio bukartii*, *Vaccinium floribundum* and *Werneria nubigena*
Depression	*Calamagrostis longiaristata*, *Scirpus* sp., *Juncus* sp., *Senecio bukartii*, *Vaccinium floribundum* and mosses
Hollow	*Scirpus* sp., *Juncus* sp. and mosses

4.5.1.3 Soil physical and chemical properties

Soil depth at the sampling stations ranged from 20 to 100 cm. Soil physical properties ranged from 0.02 to 0.28 $\mathrm{g\,m^{-3}}$ at 0 - 5 cm and 0.03 to 0.66 $\mathrm{g\,m^{-3}}$ at 5 - 15 cm for bulk density, 1.57 to 2.27 $\mathrm{g\,m^{-3}}$ at 0 - 5 cm and 1.61 to 2.57 $\mathrm{g\,m^{-3}}$ at 5 - 15 cm for particle density and subsequently 0.88 to 0.99 at 0 - 5 cm and 0.73 to 0.99 at 5 - 15 cm for porosity. Similarly, soil chemical properties ranged from 3.8 to 5.4 at 0 - 5 cm and 3.3 to 4.8 at 5 - 15 cm for pH, 13.14 to 42.43 $\%$ C at 0 -5 cm and 5.37 to 42.48 $\%$ C at 5 - 15 cm for C content and 13.14 to 36.10 at 0 - 5 cm and 10.74 to 37.89 at 5 - 15 cm for C:N. Soils on the slope and in the basin were principally delineated by soil depth, porosity, as a function of bulk and particle density, and C and N contents, with shallower, lower porosity organomineral soils on the slopes and deeper, higher porosity peat soils in the basin (Figure 4.6).

The soils on the slopes and in the basin were significantly different to each other in terms of their physical and chemical characteristics (Table 4.4). For example, the slope soils were significantly shallower with mean depths of 25.8 (1) cm and 28.8 (1) cm on the upper and lower slopes and 67.0 (14) cm and 62.3 (16) cm in the depressions and hollows, respectively. Similarly, the basin soils had significantly greater C contents than the soils on the slope with means at 0 - 5 cm depth of 38.54 (1.55) $\%$ and 40.77 (0.69) $\%$ in the depressions and hollows and 21.31 (1.30) $\%$ and 20.81 (1.55) $\%$ on

Table 4.4: Means and standard errors of soil physical and chemical properties at 0 - 5 and 5 - 15 cm for the microform groups identified in the intensive sampling campaigns. Upper case letters indicate significant differences among microform groups within a sampling depth and lower case letters indicate significant differences between depths within a microform group. [1]bulk and [2]particle density. n = 8, 9, 3, and 4 per site and depth for upper slope, lower slope, depression and hollow, respectively.

Microform	Upper slope	Lower slope	Depression	Hollow
Soil depth (cm)	25.8 (1) A	28.8 (2) A	67.0 (14) B	62.3 (16) B
BD[1], 0 - 5 cm ($g\,cm^{-3}$)	0.16 (0.01) Aa	0.15 (0.02) Aa	0.07 (0.01) Ba	0.03 (0.01) Ba
BD[1], 5 - 15 cm ($g\,cm^{-3}$)	0.49 (0.04) Ab	0.47 (0.05) Ab	0.16 (0.02) Bb	0.10 (0.04) Ba
PD[2], 0 - 5 cm ($g\,cm^{-3}$)	2.07 (0.04) Aa	2.03 (0.04) Aa	1.62 (0.03) Ba	1.82 (0.05) Ba
PD[2], 5 - 15 cm ($g\,cm^{-3}$)	2.30 (0.04) Ab	2.34 (0.05) Ab	1.68 (0.04) Ba	1.75 (0.08) Ba
Porosity, 0 - 5 cm	0.93 (0.01) Aa	0.93 (0.01) Aa	0.96 (0.00) ABa	0.98 (0.00) Ba
Porosity, 5 - 15 cm	0.79 (0.01) Ab	0.83 (0.03) ABb	0.90 (0.01) BCb	0.94 (0.02) Ca
C content, 0 - 5 cm (%)	21.31 (1.30) Aa	20.81 (1.55) Aa	38.54 (1.55) Ba	40.77 (0.69) Ba
C content, 5 - 15 cm (%)	12.60 (1.36) Ab	11.50 (1.35) Ab	35.54 (0.80) Ba	37.26 (4.54) Ba
C:N, 0 - 5 cm	14.82 (0.23) Aa	15.11 (0.45) Aa	19.95 (3.35) Aa	27.60 (3.55) Ba
C:N, 5 - 15 cm	12.29 (0.08) Ab	11.56 (0.17) Ab	14.09 (0.86) Aa	23.67 (5.40) Ba
pH, 0 - 5 cm	4.3 (0.1) Aa	4.1 (0.0) Aa	4.7 (0.3) Aa	4.5 (0.3) Aa
pH, 5 - 15 cm	3.9 (0.1) Ab	4.1 (0.1) Aa	74.0 (0.1) Aa	4.0 (0.3) Aa

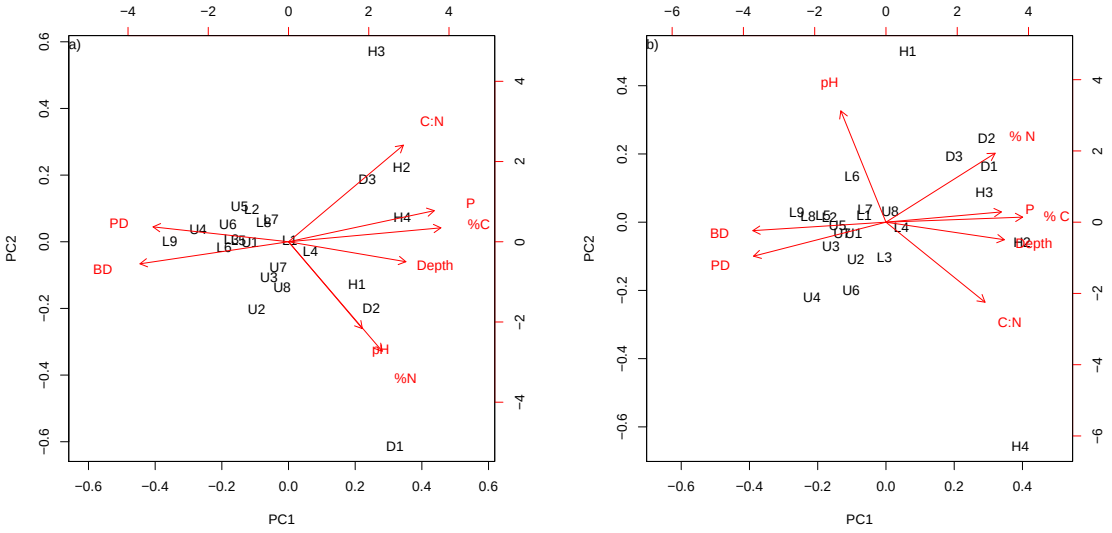

Figure 4.6: Biplots for scaled principal component analysis, where PC1 reflects the proxies (i.e. density and C content) for soil organic matter content and PC2 is related to soil N content and pH, of soil properties characterised during the intensive campaigns at upper slope (U), lower slope (L), depression (D) and hollow (H) sampling stations: a) soil conditions at 0 - 5 cm with the proportion of variance explained by PC1 and PC2 of 0.64 and 0.16, respectively and b) soil conditions at 5 - 15 cm with the proportion of variance explained by PC1 and PC2 of 0.67 and 0.16, respectively.

the upper and lower slopes, respectively. These contrasts support the inference that the basin is principally a peat forming environment, suggestive of wetland conditions, whilst upland conditions on the slopes promote the formation of organomineral soils. Deviations from this contrast between slope and basin is notable for C:N at both 0 - 5 and 5 - 15 cm with significantly greater ratios in the hollow soils, reflecting the accumulation of poorly humidified moss litter, than for slopes and depressions. Similarly, no significant differences in pH between microforms were identified across the acidic soils of this landscape.

The distinction between soil forming environments suggested by contrasts between slope and basin microforms is supported by comparison between 0 - 5 cm and 5 - 15 cm depths within microforms (Table 4.4). Soil properties are broadly homogeneous with depth within the basin microforms with significant differences between depths only apparent in greater bulk density and consequently lower porosity at 5 - 15 cm than 0 - 5 cm in the depressions. Contrastingly, significant differences between 0 - 5 cm and 5 - 15 cm depths, with denser, less organic material in deeper soils, were identified for all measured soil properties, with the exception of pH, in the slope microforms for all measured properties.

4.5.2 Soil environmental conditions

4.5.2.1 Wet season campaign

At each of the twenty four sampling stations, WFPS and water table depth, soil O_2 concentration and soil temperature were measured daily during the wet season between the 12^{th} and 22^{nd} of November 2011. During this campaign, WFPS ranged from 53.7 to 100.0 $\%$ with a median of 83.4 $\%$, water table depth ranged from 0 to 20 cm from the surface with a median of 20 cm, soil O_2 concentration ranged from 0.0 to 21.0 $\%$ with a median of 16.1 $\%$ and soil temperature ranged from 4.2 to 13.8 $^{\circ}\mathrm{C}$ with a median of 9.5 $^{\circ}\mathrm{C}$. Water table depth and soil O_2 concentration were significantly positively correlated with each other (Spearman's ρ = 0.51) and negatively correlated (Spearman's ρ = -0.39 with water table depth and -0.44 with soil O_2 concentration) with WFPS (Table 4.5).

Table 4.5: Spearman's rank correlation coefficients for correlations between water table depth (WTD), soil O_2 concentration and WFPS, CH_4 flux and CO_2 flux and soil CH_4 and CO_2 concentration for all data collected during wet and dry season campaigns. Values are Spearman's ρ (n) where negative values indicate inverse correlations and significance at $p < 0.05$ is signified by *.

Wet season	WTD	O_2 conc.	WFPS	CH_4 flux	CH_4 conc.
WTD	-	0.51 (263)*	-0.39 (256)*		
O_2 conc.	-	-	-0.44 (255)*		
CO_2 flux				0.02 (204)	
CO_2 conc.					0.80 (24)*
Dry season	**WTD**	**O_2 conc.**	**WFPS**	**CH_4 flux**	**CH_4 conc.**
WTD	-	0.53 (240)*	-0.59 (240)*		
O_2 conc.	-	-	-0.43 (240)*		
CO_2 flux				0.19 (120)*	
CO_2 conc.					-0.34 (23)

Wet season campaign means for upper slope, lower slope, depression and hollow microforms are reported in Table 4.6. WFPS was greatest in the hollows with a mean of 91.7 (0.6) $\%$ and significantly different to that of the upper slopes, lower slopes and depressions with respective means of 81.4 (1.1), 78.1 (1.0) and 80.4 (1.45) $\%$. Similarly, the water table was closest to the surface in the hollows with a mean depth of 4 (1) cm and significantly different to that of the upper slopes, lower slopes and depressions with respective means of 16 (1), 15 (1) and 17 (1) cm. Soil O_2 concentration was greatest in the depressions and upper slopes with respective means of 16.9 (0.8) and 16.8.0 (0.3)

%, followed by the lower slopes with a mean of 12.5 (0.6) % and lowest in the hollows with a mean of 0.2 (0.2) %. Soil O_2 concentration in hollows and lower slopes were significantly different to each other and that of the depressions and upper slopes. Soil temperature was greater in the upper slopes, depressions and hollows and significantly different to those of the lower slopes with respective means for upper slopes, lower slopes, depressions and hollows of 9.9 (0.2), 8.6 (0.2), 9.8 (0.3) and 9.8 (0.3) °C.

4.5.2.2 Dry season campaign

Similarly to the wet season campaign, daily measurements of WFPS and water table depth, soil O_2 concentration and soil temperature were measured daily between the 12^{th} and 21^{st} of August 2012. During this campaign, WFPS ranged from 60.6 to 100.0 % with a median of 86.4 %, water table depth ranged from 1 to 20 cm from the surface with a median of 20 cm, soil O_2 concentration ranged from 0.0 to 21.0 % with a median of 19.9 % and soil temperature ranged from 4.6 to 11.3 °C with a median of 7.2 °C. Water table depth and soil O_2 concentration were significantly positively correlated with each other (Spearman's ρ = 0.53) and negatively correlated (Spearman's ρ = -0.59 with water table depth and -0.43 with soil O_2 concentration) with WFPS (Table 4.5).

Dry season campaigns means for upper slope, lower slope, depression and hollow microforms are reported in Table 4.6. WFPS was highest, with a mean of 96.0 (0.7) % in hollows and lowest, with a mean of 75.8 (1.7) %, in the depressions. WFPS in the depressions and hollows was significantly different from each other and that of the upper and lower slopes where respective means were 84.2 (1.1) % and 84.1 (1.1) %. The water table was furthest from the surface in the depressions, with a mean depth of 20 (0) cm, followed by the upper slopes, with a mean depth of 19 (0) cm, and lower slopes, with a mean depth of 17 (1) cm. The water table was closest to the surface in the hollows with a mean depth of 13 (1) cm. Water table depth in the hollows was significantly different to those of the depressions and slopes, as were those of the depressions and lower slopes. Soil O_2 concentration was greatest in the depressions and upper slopes, with respective means of 20.1 (0.1) and 20.0 (0.1) %, and significantly different to the lower concentrations of 18.7 (0.3) and 16.9 (0.9) % for the lower slopes

Table 4.6: Means and standard errors of flux rates and environmental conditions for the microform groups identified in the intensive sampling campaigns. Upper case letters indicate significant differences among microform groups within a campaign. n = 8, 9, 3, and 4 per microform and day for upper slope, lower slope, depression and hollow, respectively.

Campaign -	Microform -	Net CH_4 flux (mg CH_4-C m^{-2} d^{-1})	Net CO_2 flux (g CO_2-C m^{-2} d^{-1})	O_2 concentration (%)	WFPS (%)	Water table depth (cm)	Soil temperature (°C)
dry	Upper slope	-0.45 (0.12) A	1.30 (0.06) A	20.0 (0.1) A	84.2 (1.1) A	19 (0) AB	6.9 (0.1) A
dry	Lower slope	1.43 (0.35) B	1.48 (0.74) A	18.7 (0.3) B	84.1 (1.1) A	17 (1) B	7.0 (0.1) A
dry	Depression	-0.64 (0.06) A	2.38 (0.26) B	20.1 (0.1) A	75.8 (1.7) B	20 (0) A	7.8 (0.2) B
dry	Hollow	16.26 (1.28) C	2.08 (0.21) B	16.6 (0.9) B	96.0 (0.7) C	13 (1) C	8.4 (0.2) B
wet	Upper slope	0.22 (0.12) A	1.74 (0.06) A	16.8 (0.3) A	81.4 (1.1) A	16 (1) A	9.9 (0.2) A
wet	Lower slope	7.45 (1.31) B	2.06 (0.08) B	12.5 (0.6) B	78.1 (1.0) A	15 (1) A	8.6 (0.2) B
wet	Depression	-0.23 (0.10) C	3.53 (0.22) C	16.9 (0.8) A	80.4 (1.5) A	17 (1) A	9.8 (0.3) A
wet	Hollow	108.19 (10.32) D	2.38 (0.18) B	0.2 (0.2) C	91.7 (0.6) B	4 (1) B	9.8 (0.3) A

and hollows, respectively. Soil temperatures were higher in the basin features and significantly different to those of the slopes with respective means for upper slopes, lower slopes, depressions and hollows of of 6.9 (0.1), 7.0 (0.1), 7.8 (0.2) and 8.4 (0.2) ℃.

4.5.3 Soil-atmosphere exchange and soil concentration of CH_4 and CO_2

4.5.3.1 Wet season campaign

At each of the twenty four sampling stations, static flux chamber measurements were made to determine net CH_4 and CO_2 flux rates daily between the 12^{th} and 22^{nd} of November 2011. On the final day of the campaign, soil gas equilibration chambers were sampled to determine soil gas concentrations of CH_4 and CO_2. During this campaign, net CH_4 fluxes ranged from -1.94 to 284.80 mg CH_4-C m^{-2} d^{-1} with a median of 1.24 mg CH_4-C m^{-2} d^{-1}. Of the 264 static flux chamber measurements for CH_4 made during this campaign 11 were excluded owing to methodological failures. Of the remaining 253 measurements, 63 % of measurements were emission, 18 % of measurements were uptake and 19 % of measurements had no detectable flux. Fluxes of CO_2 ranged from 0.18 to 7.47 g CO_2-C m^{-2} d^{-1} with a median of 1.99 g CO_2-C m^{-2} d^{-1}. Wet season fluxes of CH_4 and CO_2 were not significantly correlated (Table 4.5). Soil gas concentrations of CH_4 ranged from 2.26 to 61,100 ppm with a median of 119 ppm and CO_2 concentrations ranged from 0.21 to 8.34 % with a median of 2.26 %. Wet season soil concentrations of these gases were significantly positively correlated (Spearman's ρ = 0.80).

Wet season campaign means for CH_4 and CO_2 fluxes from the upper slopes, lower slopes, depressions and hollows are reported in Table 4.6. Wet season net CH_4 fluxes were significantly different between all microform groups, with respective means for the upper slopes, lower slopes, depressions and hollows of 0.22 (0.12), 7.45 (1.31), -0.23 (0.10) and 108.19 (10.32) mg CH_4-C m^{-2} d^{-1}. The distribution of wet season CH_4 fluxes between emission, uptake and no flux for the upper slopes, lower slopes, depressions and hollows are reported in Table 4.7. In the upper slopes fluxes were dominated

by marginal activity with no fluxes accounting for 44.05 % of measurements, whilst, emission and uptake accounted for the remaining 34.52 % and 21.43 %, respectively. Sink activity dominated in the depressions with 64.52 % of measurements accounted for by uptake, whilst, emissions and no fluxes accounted for the remaining 9.68 % and 25.81 % , respectively. Fluxes from the lower slopes and hollows were dominated by emission activity, respectively, accounting for 89.36 % and 100.00 % of measurements. In the lower slopes, uptake and no fluxes were detected in 7.45 % and 3.19 % of cases, respectively. Similarly, to observations from the long-term monthly measurements, spatial variations between sampling locations within microform groupings were of a similar order of magnitude to between sampling day variations. Wet season CO_2 fluxes were lowest from the upper slopes with a mean of 1.74 (0.06) g CO_2-C m^{-2} d^{-1} and greatest from the depressions with a mean of 3.53 (0.22) g CO_2-C m^{-2} d^{-1}. Fluxes from the upper slopes and depressions were significantly different to both each other and those from the lower slopes and hollows where respective means were 2.06 (0.08) and 2.38 (0.18) g CO_2-C m^{-2} d^{-1}. Soil CH_4 concentrations were highly variable within microforms and were greatest in the hollows, with a mean of 42700 (8100) ppm, and significantly different to those of the upper slopes, lower slopes and depressions where respective means were 82.3 (48.5), 3160 (1540), and 8.04 (3.04) ppm (Table 4.8). Soil CO_2 concentrations were lowest in the depressions with a mean of 1.07 (0.27) % and significantly different to that of the hollows with a mean of 4.41 (1.08) %. No significant differences were identified between the slope and basin features, with means of 1.63 (0.49) and 3.40 (0.94) % for the upper and lower slopes, respectively.

4.5.3.2 Dry season campaign

Similarly to the wet season campaign, static flux chamber measurements were made between the 12^{th} and 21^{st} of August 2012. On the final day of the campaign soil gas equilibration chambers were sampled to determine soil gas concentrations of CH_4 and CO_2. During this campaign, net CH_4 fluxes ranged from -2.04 to 38.81 mg CH_4-C m^{-2} d^{-1} with a median of 0.37 mg CH_4-C m^{-2} d^{-1}. For net CH_4 fluxes, of the 240 static flux chamber measurements made during this campaign 21 were excluded owing to

Table 4.7: Proportions of net CH_4 flux measurements accounted for by methodological failures, indeterminable or zero fluxes, emission fluxes and uptake fluxes by microform group for each intensive campaign. Values are % and number of observations. Percentages of failures are calculated from the total number of observations. Whilst failures are excluded in calculating percentages of zero, emission and uptake fluxes.

Campaign	Microform	Fail	Zero	Emission	Uptake
dry	Upper slope	3.90 (3)	67.53 (52)	6.49 (5)	25.97 (20)
dry	Lower slope	7.14 (6)	42.86 (36)	51.19 (43)	5.95 (5)
dry	Depression	30.43 (7)	30.43 (7)	0.00 (0)	69.57 (16)
dry	Hollow	14.29 (5)	0.00 (0)	100.00 (35)	0.00 (0)
wet	Upper slope	4.76 (4)	44.05 (37)	34.52 (29)	21.43 (18)
wet	Lower slope	5.32 (5)	3.19 (3)	89.36 (84)	7.45 (7)
wet	Depression	6.45 (2)	25.81 (8)	9.68 (3)	64.52 (20)
wet	Hollow	0.00 (0)	0.00 (0)	100.00 (44)	0.00 (0)

methodological failures. Of the remaining 219 measurements, 38 % of measurements were emission, 19 % of measurements were uptake and 43 % of measurements had no detectable flux. Fluxes of CO_2 ranged from 0.43 to 6.75 g CO_2-C m^{-2} d^{-1} with a median of 1.44 g CO_2-C m^{-2} d^{-1}. Dry season net fluxes of CH_4 and CO_2 were significantly positively correlated (Spearman's ρ = 0.19) (Table 4.5). Soil gas concentrations of CH_4 ranged from 2.38 to 1,150 ppm with a median of 13.2 ppm and CO_2 concentrations ranged from 0.15 to 2.23 % with a median of 0.93 %. There was no significant correlation between these gases during the dry season.

Table 4.8: Mean and standard errors of soil CH_4 and CO_2 concentrations by microform for intensive campaign. Upper case letters indicate significant differences among microforms within a campaign. n = 8, 9 3, and 4 per microform for upper slope, lower slope, depression and hollow, respectively.

Campaign -	Microform -	CH_4 concentration (ppm)	CO_2 concentration (%)
dry	Upper slope	16.9 (2.3) A	0.48 (0.16) A
dry	Lower slope	11.7 (2.3) A	1.37 (0.19) B
dry	Depression	57.0 (54.2) A	0.56 (0.18) A
dry	Hollow	351 (272) A	1.18 (0.39) AB
wet	Upper slope	82.3 (48.5) A	1.63 (0.49) AB
wet	Lower slope	3160 (1540) A	3.4 (0.94) AB
wet	Depression	8.04 (3.04) A	1.07 (0.27) A
wet	Hollow	42700 (8100) B	4.41 (1.08) B

Dry season campaign means for net CH_4 and CO_2 fluxes from the upper slopes, lower slopes, depressions and hollows are reported in Table 4.6. Dry season net CH_4 fluxes were lowest from the upper slopes and depressions with means of -0.45 (0.12) and -

0.64 (0.06) mg CH_4-C m^{-2} d^{-1}. Larger fluxes from the lower slopes and hollows were significantly different to these and each other with respective means of 1.43 (0.35) and 16.26 (1.28) mg CH_4-C m^{-2} d^{-1}. The distribution of dry season net CH_4 fluxes between emission, uptake and no flux for the upper slopes, lower slopes, depressions and hollows are reported in Table 4.7. In the upper slopes fluxes were dominated by marginal activity with no fluxes detected for 67.53 % of measurements, whilst, emission and uptake accounted for 6.49 % and 25.97 %, respectively. Sink activity dominated in the depressions with 69.57 % of measurements accounted for by uptake, whilst, emissions and no fluxes accounted for 0.00 % and 30.43 %, respectively. Fluxes from the lower slopes were dominated by emissions and no flux activity with these accounting, respectively, for 51.19 % and 42.86 % of measurements, whilst, uptake accounted for 5.95 %. Hollows acted solely as a source with emissions accounting for 100 % of measurements. Similarly to the wet season campaign, variations in net CH_4 fluxes within microform were larger between sampling stations than between sampling days. Dry season CO_2 fluxes from the slopes were lower and significantly different to those of the basin features with respective means for the upper slopes, lower slopes, depressions and hollows of 1.30 (0.06), 1.48 (0.74), 2.38 (0.26) and 2.08 (0.21) g CO_2-C m^{-2} d^{-1}. There were no significant differences, reflecting considerable spatial variability, in soil CH_4 concentrations between microform types with respective means for upper slopes, lower slopes, depressions and hollows of 16.9 (2.3), 11.7 (2.3), 57.0 (54.2), 351 (272) ppm (Table 4.8). Soil CO_2 concentrations had respective means for upper slopes, lower slopes, depressions and hollows of 0.48 (0.16), 1.37 (0.19), 0.56 (0.18) and 1.18 (0.39) %. Soil CO_2 concentrations in the lower slopes were significantly different to those of the upper slopes and depressions.

4.5.4 Spatial relationships between net CH_4 flux and soil conditions

Across the landscape, the sampling station mean of wet and dry seasonal campaign net CH_4 fluxes was significantly negatively correlated with elevation (Spearman's ρ = -0.55), above ground biomass of *Calamagrostis longiaristata* (Spearman's ρ = -0.56)

and bulk density at 0 - 5 cm (Spearman's ρ = -0.41) and postively correlated with above ground biomass of *Juncus* sp. (Spearman's ρ = 0.45), porosity at 0 - 5 cm (Spearman's ρ = 0.42) and C:N at 0 - 5 cm (Spearman's ρ = 0.45) (Table 4.9). However, these relationships poorly characterise differences in fluxes across the basin sampling stations. Among slope sampling stations net CH_4 flux was significantly negatively correlated with elevation (Spearman's ρ = -0.82), C:N at 5 - 15 cm (Spearman's ρ = -0.70) and positively correlated with down slope angle (Spearman's ρ = 0.53) and pH at 5 - 15 cm (Spearman's ρ = 0.65) (Table 4.9). Among basin features, mean net CH_4 flux was positively correlated with particle density at 0 - 5 cm (Spearman's ρ = 0.79) and negatively related to N content at 5 - 15 cm (Spearman's ρ = -0.82) (Table 4.9).

Table 4.9: Spearman's rank correlation coefficients for significant correlations between net CH_4 flux and edaphic conditions across the landscape (n = 24), slope (n = 17) and basin (n = 7). Values are Spearman's ρ where negative values indicate inverse correlations, significance at $p < 0.05$ is signified by *. Landscape: [1] above ground biomass of Calamagrostis, [2] above ground biomass of Juncus, [3] bulk density between 0 - 5 cm, [4] porosity between 0 - 5 cm and [5] C:N between 0 - 5 cm, Slope: [6] downslope angle (NE-SW), [7] C:N between 5 - 15 cm and [8] pH between 5 - 15 cm and Basin: [9] particle density between 0 - 5 cm, [10] N content between 5 - 15 cm.

Landscape	CH_4 flux	Elevation	Cala.[1]	Junc.[2]	BD[3]	P[4]	C:N[5]
CH_4 flux	-	-0.55*	-0.56*	0.45*	-0.41*	0.42*	0.45*
Elevation	-	-	0.51*	-0.51*	0.61*	-0.61*	-0.58*
Cala.[1]	-	-	-	-0.64*	0.60*	-0.60*	-0.48*
Junc.[2]	-	-	-	-	-0.33	0.34	0.28
BD[3]	-	-	-	-	-	-0.99*	-0.86*
P[4]	-	-	-	-	-	-	-0.77*
Slope	**CH_4 flux**	**Elevation**	**NE-SW angle[6]**	**C:N[7]**	**pH[8]**		
CH_4 flux	-	-0.82*	0.53*	-0.70*	0.65*		
Elevation	-	-	-0.61*	0.64*	-0.64*		
NE-SW angle[6]	-	-	-	-0.53*	0.38		
C:N[7]	-	-	-	-	-0.67*		
Basin	**CH_4 flux**	**PD[9]**	**N content[10]**				
CH_4 flux	-	0.79*	-0.82*				
PD[9]	-	-	-0.89*				

Across the landscape, wet season campaign sampling station mean net CH_4 flux was significantly negatively correlated with soil O_2 concentration (Spearman's ρ = -0.84) and water table depth (Spearman's ρ = -0.49) and positively correlated with WFPS (Spearman's ρ = 0.47) (Figure 4.7 and Table 4.10). Similarly for the dry season campaign, net CH_4 flux was significantly negatively correlated with soil O_2 concentration

Table 4.10: Spearman's rank correlation coefficients for correlations between net CH_4 flux, water table depth (WTD), WFPS, soil O_2, CH_4 and CO_2 concentrations across the landscape (n = 24), slope (n = 17) and basin (n = 7) for wet and dry season campaigns. Values are Spearman's ρ where negative values indicate inverse correlations and significance at $p < 0.05$ is signified by *.

Landscape Wet season	CH_4 flux	WTD	WFPS	O_2 conc.	CH_4 conc.	CO_2 conc.
CH_4 flux	-	-0.49*	0.47*	-0.84*	0.79*	0.63*
WTD	-	-	-0.31	0.43*	-0.42*	-0.22
WFPS	-	-	-	-0.66*	0.57*	0.41*
O_2 conc.	-	-	-	-	-0.85*	-0.81*
CH_4 conc.	-	-	-	-	-	0.80*
Dry season	**CH_4 flux**	**WTD**	**WFPS**	**O_2 conc.**	**CH_4 conc.**	**CO_2 conc.**
CH_4 flux	-	-0.57*	0.59*	-0.81*	0.15	0.70*
WTD	-	-	-0.60*	0.65*	-0.11	-0.44*
WFPS	-	-	-	-0.51*	0.28	0.23
O_2 conc.	-	-	-	-	0.05	-0.78*
CH_4 conc.	-	-	-	-	-	-0.34
Slope Wet season	CH_4 flux	WTD	WFPS	O_2 conc.	CH_4 conc.	CO_2 conc.
CH_4 flux	-	-0.22	0.02	-0.75*	0.67*	0.59*
WTD	-	-	0.10	0.14	-0.10	-0.07
WFPS	-	-	-	-0.32	0.26	0.30
O_2 conc.	-	-	-	-	-0.87*	-0.90*
CH_4 conc.	-	-	-	-	-	0.88*
Dry season	**CH_4 flux**	**WTD**	**WFPS**	**O_2 conc.**	**CH_4 conc.**	**CO_2 conc.**
CH_4 flux	-	-0.20	0.19	-0.81*	-0.24	0.79*
WTD	-	-	-0.25*	0.51*	0.20	-0.41
WFPS	-	-	-	-0.24	0.01	0.07
O_2 conc.	-	-	-	-	0.44	-0.85*
CH_4 conc.	-	-	-	-	-	-0.44
Basin Wet season	CH_4 flux	WTD	WFPS	O_2 conc.	CH_4 conc.	CO_2 conc.
CH_4 flux	-	-0.86*	0.96*	-0.14	0.68	-0.03
WTD	-	-	-0.82*	-0.07	-0.54	-0.09
WFPS	-	-	-	-0.21	0.57	0.20
O_2 conc.	-	-	-	-	-0.07	-0.09
CH_4 conc.	-	-	-	-	-	-0.71
Dry season	**CH_4 flux**	**WTD**	**WFPS**	**O_2 conc.**	**CH_4 conc.**	**CO_2 conc.**
CH_4 flux	-	-0.94*	0.96*	-0.96*	0.46	0.54
WTD	-	-	-0.93*	0.93*	-0.56	-0.58
WFPS	-	-	-	-1.00*	0.61	0.60
O_2 conc.	-	-	-	-	-0.61	0.60
CH_4 conc.	-	-	-	-	-	0.09

(Spearman's ρ = -0.81) and water table depth (Spearman's ρ = -0.57) and positively correlated with WFPS (Spearman's ρ = 0.59). Wet season campaign mean net CH_4 flux was significantly positively correlated with soil CH_4 (Spearman's ρ = 0.79) and CO_2 (Spearman's ρ = 0.63) concentrations (Figure 4.8 and Table 4.10). In contrast the dry season campaign mean net CH_4 flux was significantly positively correlated with soil CO_2 concentration (Spearman's ρ = 0.70) but not soil CH_4 concentration. Among slope sampling stations, mean net CH_4 flux was significantly negatively correlated with soil O_2 concentration for both the wet (Spearman's ρ = -0.75) and dry (Spearman's ρ = -0.81) season campaigns. Wet season campaign mean net CH_4 flux was positively correlated with soil CH_4 (Spearman's ρ = 0.67) and CO_2 (Spearman's ρ = 0.59) concentrations. However dry season campaign mean net CH_4 flux was significantly positively correlated with soil CO_2 concentration (Spearman's ρ = 0.79) but not soil CH_4 concentration. Among basin sampling stations, wet season campaign mean net CH_4 flux was significantly negatively correlated with water table depth (Spearman's ρ = -0.86) and positively correlated with WFPS (Spearman's ρ = 0.96) but no significant relationship was found with soil O_2 concentration. Similarly, for the dry season net CH_4 flux was significantly negatively correlated with soil O_2 concentration (Spearman's ρ = -0.96) and water table depth (Spearman's ρ = -0.93) and positively with WFPS (Spearman's ρ = 0.96). Basin sampling station mean net CH_4 fluxes were not significantly correlated with soil CH_4 or CO_2 concentration in either campaign.

4.5.5 In vitro gross process rates

4.5.5.1 Observed and modelled rates of in vitro CH_4 cycling

Following the wet season intensive sampling campaign, soils from 0 - 5 and 5 - 15 cm within collar footprints at each sampling station were sampled to investigate in vitro gross rates of CH_4 production and consumption under an oxic headspace and field soil moisture conditions using an isotope pool dilution approach. Whilst the incubation temperature of 24 $^{\circ}\mathrm{C}$ is approximately double the mean annual temperature for the field site this treatment was common among the incubations. As such, the measured

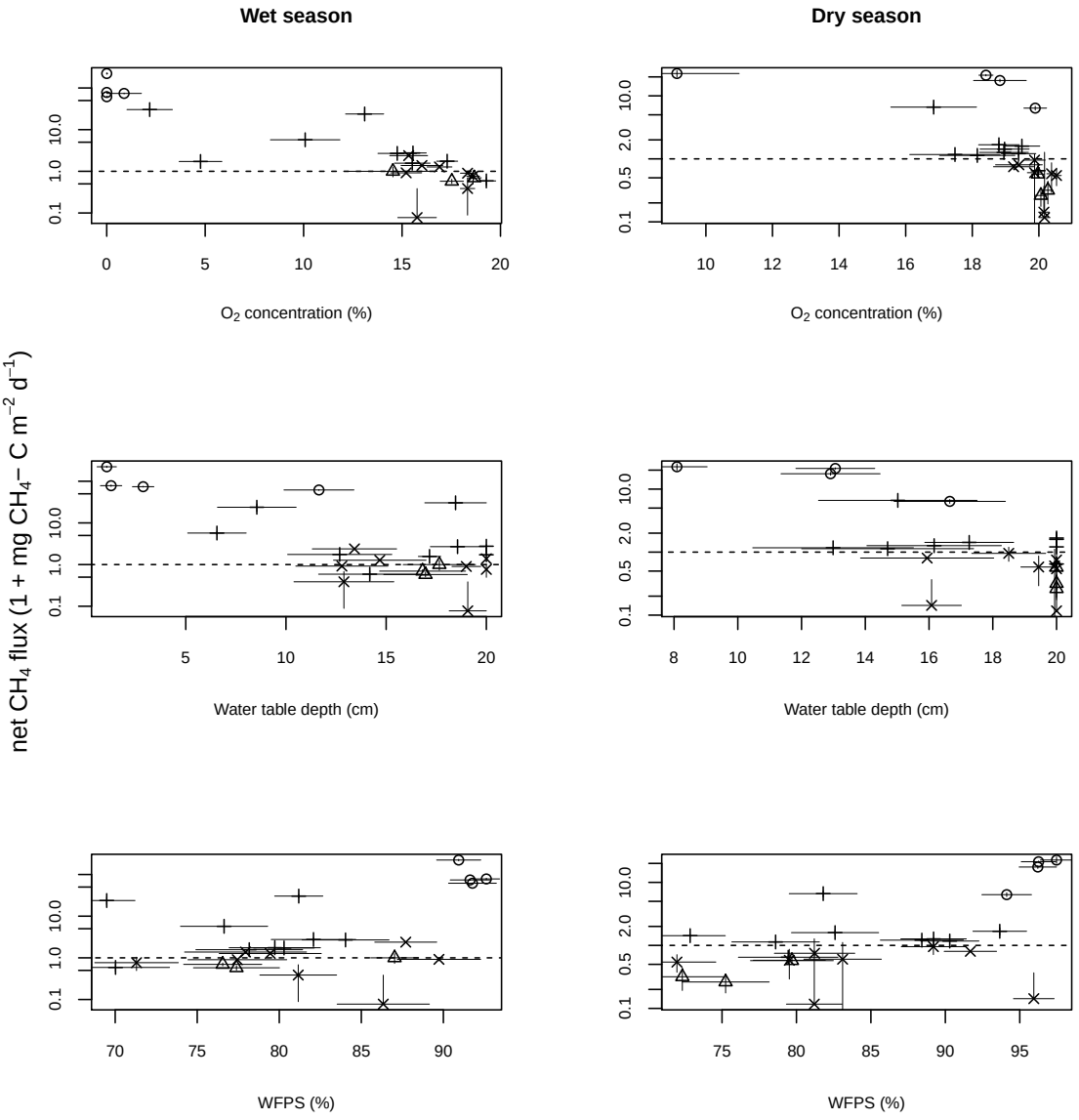

Figure 4.7: Relationships between wet (a,b and c) and dry (d,e and f) season campaign sampling station means of net CH_4 flux and soil O_2 concentration, water table depth and WFPS on a log(+1)-normal scale to aid visualisation (n = 11 in wet season and 10 in dry season). Error bars are standard errors of campaign measurements. Upper slope sampling stations are indicated by ×, lower slope by + , depression by △ and hollows by ∘. The dashed horizontal line indicates the flux equivalent to 0 mg CH_4-C m^{-2} d^{-1}.

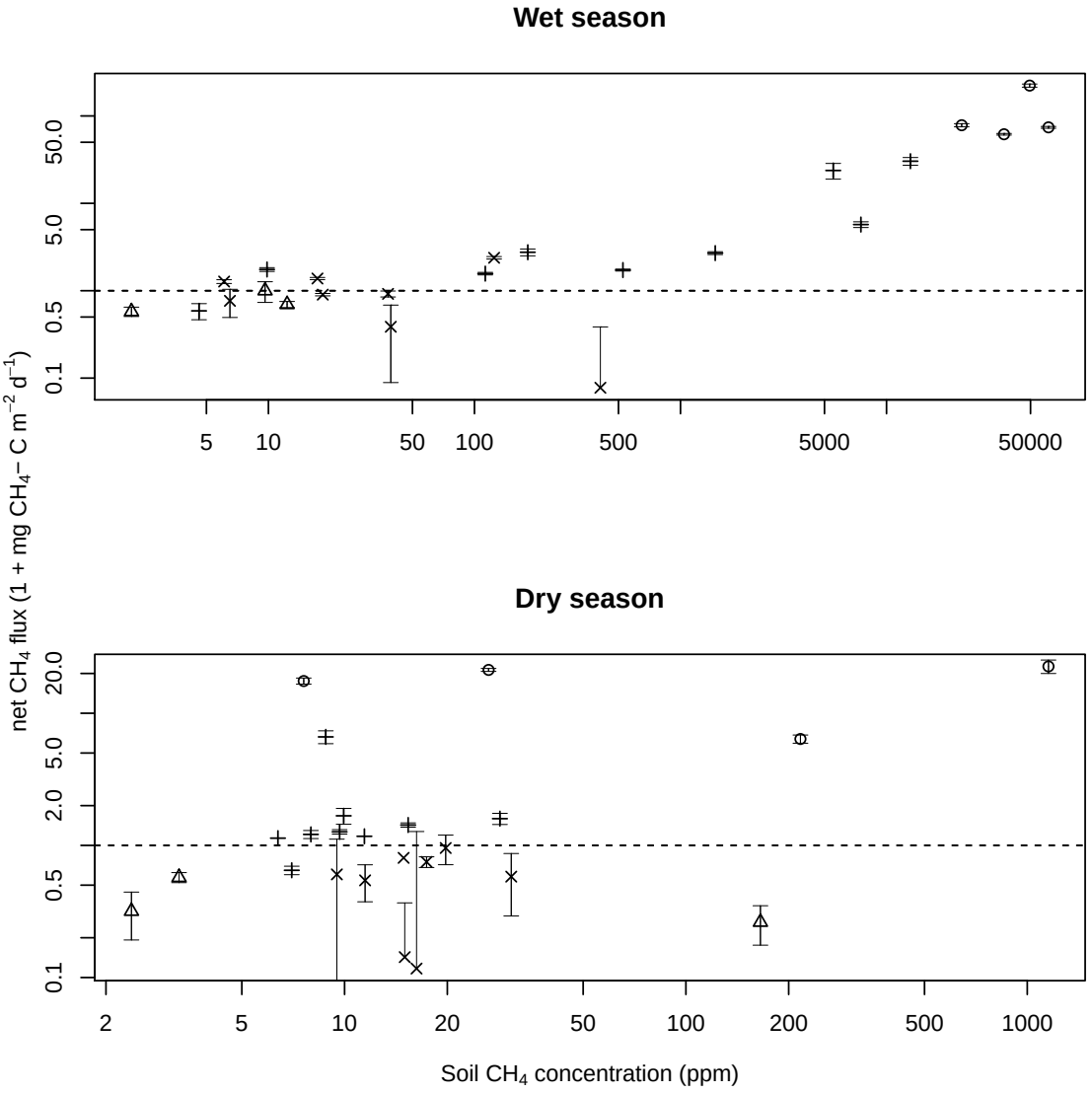

Figure 4.8: Relationships between wet and dry season campaign sampling station means of net CH_4 flux (n = 11 in wet season and 10 in dry season) and campaign soil CH_4 concentration on a log(+1)-log scale to aid visualisation. Net CH_4 flux error bars are standard errors of campaign measurements. Upper slope sampling stations are indicated by ×, lower slope by + , depression by △ and hollows by ∘. The dashed horizontal line indicates the flux equivalent to 0 mg CH_4-C m^{-2} d^{-1}.

rates of CH_4 cycling may not be realistic of those found in the field but we are able to investigate the relative influence of soil moisture and properties. Observed net fluxes of CH_4 from 0 - 5 cm were highly variable within microforms types with means of 3.28 (1.39) µg CH_4-C g dry soil^{-1} d^{-1} for the upper slopes, 24.30 (17.93) µg CH_4-C g dry soil^{-1} d^{-1} for the lowers slopes, 42.95 (40.62) µg CH_4-C g dry soil^{-1} d^{-1} for the depressions and 4.46 (10.35) µg CH_4-C g dry soil^{-1} d^{-1} for the hollows (Table 4.11). Reflecting this variability no significant differences between microforms were identified. Variability in net CH_4 fluxes in these incubations resulted from high rates of net CH_4 production in some soil samples. In ten of these instances, accounting for one hollow, two depression, four lower slope and three upper slope incubations these rates of production, with observed net CH_4 fluxes ranging from 4.38 to 164.02 µg CH_4-C g dry soil^{-1} d^{-1}, were such that the assumptions of the isotope pool dilution model applied to deconvolve gross rates of consumption and production were violated. As such, rates of production and consumption were not modelled for these cases. Exclusion of these incubations considerably reduced the range of net emissions observed with means of 1.13 (1.51) µg CH_4-C g dry soil^{-1} d^{-1} for the upper slopes, 0.19 (0.64) µg CH_4-C g dry soil^{-1} d^{-1} for the lower slopes, -2.89 (NA) µg CH_4-C g dry soil^{-1} d^{-1} for the remaining depression incubation and -5.49 (4.01) µg CH_4-C g dry soil^{-1} d^{-1} for the hollows. In contrast, net fluxes in incubations of soils from 5 - 15 cm were more uniform within microforms with means of -1.07 (0.24) µg CH_4-C g dry soil^{-1} d^{-1} for the upper slopes, -0.75 (0.11) µg CH_4-C g dry soil^{-1} d^{-1} for the lower slopes, -2.05 (0.11) µg CH_4-C g dry soil^{-1} d^{-1} for the depressions and -5.51 (1.74) µg CH_4-C g dry soil^{-1} d^{-1} for the hollows . Observed net CH_4 fluxes were significantly smaller in incubations of basin than the slope soils. Within microforms, net fluxes of CH_4 were only significantly different between 0 - 5 cm and 5 - 15 cm for the incubations of upper slope soils. In incubations of soils from both 0 - 5 and 5 - 15 cm, observed CO_2 fluxes were significantly greater in the hollows than those of the depression and slope soils. Similarly, fluxes in incubations of depression soils were significantly greater than those of slope soils. For all microforms gross rates of CO_2 fluxes were significantly greater at 0 - 5 cm than 5 - 15 cm with respective means of 17.98 (2.05) and 2.83 (0.56) mg CO_2-C g dry soil^{-1} d^{-1} for the upper slopes, 17.59 (2.67) and 3.28 (0.46) mg CO_2-C g dry soil^{-1}

d^{-1} for the lower slopes, 43.36 (3.89) and 9.70 (1.34) mg CO_2-C g dry $soil^{-1}$ d^{-1} for the depressions and 76.21 (10.76) and 26.72 (6.25) mg CO_2-C g dry $soil^{-1}$ d^{-1} for the upper slopes.

The model represented a good fit to the data with r^2 of 0.96 and 0.94 for linear regressions between observed and modelled headspace concentration of CH_4 and observed and modelled headspace atom $\%$ of ^{13}C - CH_4, respectively (Figure 4.9). Modelled rates of gross CH_4 production and consumption tended to overestimate net flux of CH_4 but represent a reasonable approximation of variations across incubations with an r^2 for the linear regression between observed and modelled net fluxes of 0.78. Model estimates of gross rates of CH_4 consumption and production and rates of C mineralisation are reported in Table 4.12. Modelled gross rates of CH_4 consumption at 0 - 5 cm were 6.34 (0.90) µg CH_4-C g dry $soil^{-1}$ d^{-1} for upper slopes, 2.56 (0.62) µg CH_4-C g dry $soil^{-1}$ d^{-1} for lower slopes, 9.85 (NA) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the depression and 24.13 (9.73) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the hollows. Rates of consumption were significantly greater in incubations of upper than lower slope soils at this depth, whilst there was considerable variability for incubations of hollow soils. Mean rates of gross consumption for soils from 5 - 15 cm were 3.30 (0.51) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the upper slopes, 2.59 (0.35) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the lower slopes, 8.02 (0.63) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the depressions and 17.30 (4.43) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the hollows. Gross rates of consumption were significantly greater in incubations of basin than slope soils at this depth. Significant differences between gross rates of consumption at 0 - 5 cm and 5 - 15 cm were only identified for incubations of soils from the upper slopes. Mean modelled gross rates of CH_4 production in incubations of soils from 0 - 5 cm were 9.46 (1.36) µg CH_4-C g dry $soil^{-1}$ d^{-1} for upper slopes, 3.21 (0.46) µg CH_4-C g dry $soil^{-1}$ d^{-1} for lower slopes, 8.11 (NA) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the depression and 21.03 (4.36) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the hollows. There were significant differences among incubations of upper slope, lower slope and hollow soils at this depth. In incubations of soils from 5 - 15 cm gross rates of production had means of 2.66 (0.38) µg CH_4-C g dry $soil^{-1}$ d^{-1} for upper slopes, 2.02 (0.30) µg CH_4-C g dry $soil^{-1}$ d^{-1}, 7.16 (0.14) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the depression and 11.88 (4.43) µg CH_4-C g dry $soil^{-1}$ d^{-1} for the hollows. Gross rates of

Table 4.11: Mean and standard errors of GWC (gravimetric water content) and in vitro observed net CH_4 and CO_2 fluxes for soils incubated from 0 - 5 cm and 5 - 15 cm by microform. n = 8, 9, 3, and 4 per microform and depth for upper slope, lower slope, depression and hollow, respectively. Capital letters indicate significant differences among microform groups within depth and lower case letters indicate significant differences within microforms between depths.

Microform -	Depth (cm)	No. incubations	GWC -	Observed net CH_4 flux (μg CH_4-C g dry soil^{-1} d^{-1})	Observed net CO_2 flux (mg CO_2-C g dry soil^{-1} d^{-1})
Upper slope	0 - 5	8	0.81 (0.02) Aa	3.28 (1.39) Aa	17.98 (2.05) Aa
Lower slope	0 - 5	9	0.82 (0.02) Aa	24.30 (17.93) Aa	17.59 (2.67) Aa
Depression	0 - 5	3	0.93 (0.01) Ba	42.95 (40.62) Aa	43.36 (3.89) Ba
Hollow	0 - 5	4	0.96 (0.01) Ca	4.46 (10.35) Aa	76.21 (10.76) Ca
Upper slope	5 - 15	8	0.64 (0.03) Ab	-1.07 (0.24) Ab	2.83 (0.56) Ab
Lower slope	5 - 15	9	0.63 (0.03) Ab	-0.75 (0.11) Aa	3.28 (0.46) Ab
Depression	5 - 15	3	0.85 (0.01) Bb	-2.05 (0.11) Ba	9.70 (1.34) Bb
Hollow	5 - 15	4	0.91 (0.04) Ba	-5.51 (1.74) Ba	26.72 (6.25) Cb

production were significantly greater in incubations of basin than slope soils from this depth. Gross rates of production were significantly lower in at 5 - 15 cm than 0 - 5 cm in incubations of upper slope soils.

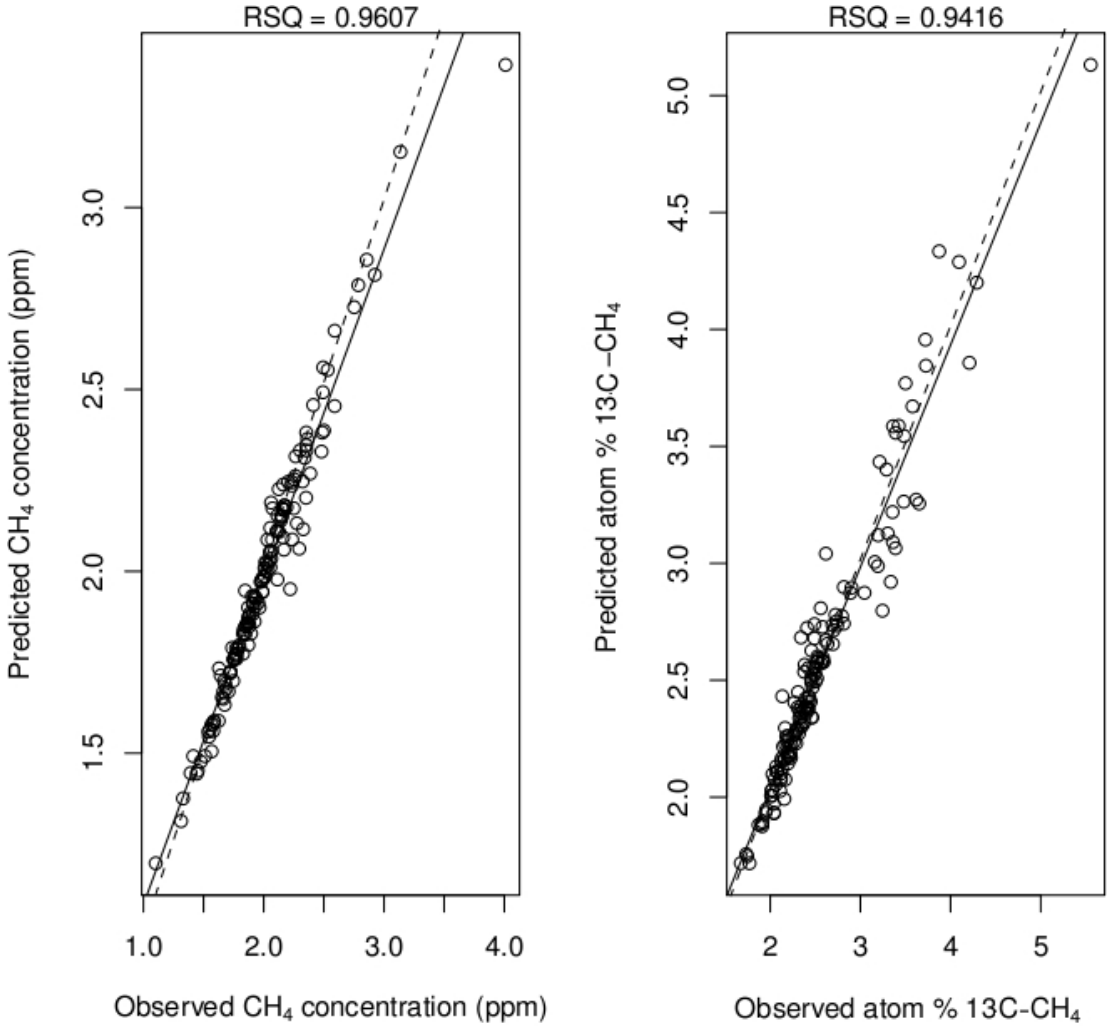

Figure 4.9: Pooled comparison of predicted against observed CH_4 concentration and atom % (n = 112). Fitted linear relationships and 1:1 lines between predicted and observed values are indicated by solid and dashed lines, respectively.

Rates of total C mineralisation reflected patterns in observed CO_2 fluxes. For incubations of soils from 0 - 5 cm mean rates of total C mineralisation were 18.78 (3.09) mg C g dry soil^{-1} d^{-1} for the upper slopes, 11.71 (1.61) mg C g dry soil^{-1} d^{-1} for the lower slopes, 51.12 (NA) mg C g dry soil^{-1} d^{-1} for the depression and 81.10 (13.57) mg C g dry soil^{-1} d^{-1} for the hollows. Total C mineralisation was significantly greater in incubations of hollow soils than those of the slopes at this depth. Similarly, in incubations of soils from 5 - 15 cm rates of total C mineralisation were 2.84 (0.56) mg C

Table 4.12: Mean and standard errors of in vitro modelled rates of gross CH_4 cycling and soil C mineralisation for soils incubated from 0 - 5 cm and 5 - 15 cm by microform groups. n = 5, 5, 1, and 3 per microform and depth for upper slope, lower slope, depression and hollow, respectively. Capital letters indicate significant differences among microform groups within depth and lower case letters indicate significant differences within microforms between depths.

Microform -	Depth cm	No. incubations	Modelled CH_4 consumption (µg CH_4-C g dry soil^{-1} d^{-1})	Modelled CH_4 production (µg CH_4-C g dry soil^{-1} d^{-1})	Total C mineralisation (mg C g dry soil^{-1} d^{-1})	Methanogenic fraction -
Upper slope	0 - 5	5	6.34 (0.90) Aa	9.46 (1.36) Aa	18.78 (3.09) Aa	0.11 (0.01) Aa
Lower slope	0 - 5	5	2.56 (0.62) Ba	3.21 (0.46) Ba	11.71 (1.61) Aa	0.06 (0.01) Ba
Depression	0 - 5	1	9.85 (NA)	8.11 (NA)	51.12 (NA)	0.03 (NA)
Hollow	0 - 5	3	24.13 (9.73) ABa	21.03 (4.36) Ca	81.10 (13.57) Ba	0.05 (0.01) Ba
Upper slope	5 - 15	8	3.30 (0.51) Ab	2.66 (0.38) Ab	2.84 (0.56) Ab	0.27 (0.09) ABa
Lower slope	5 - 15	9	2.59 (0.35) Aa	2.02 (0.30) Aa	3.28 (0.46) Ab	0.15 (0.04) ABb
Depression	5 - 15	3	8.02 (0.63) B	7.16 (0.14) B	9.70 (1.34) B	0.15 (0.02) A
Hollow	5 - 15	4	17.30 (4.43) Ba	11.88 (2.48) Ba	26.73 (6.25) Cb	0.09 (0.01) Bb

g dry soil^{-1} d^{-1} for the upper slopes, 3.28 (0.46) mg C g dry soil^{-1} d^{-1} for the lower slopes, 9.70 (1.34) mg C g dry soil^{-1} d^{-1} for the depressions and 26.73 (6.25) mg C g dry soil^{-1} d^{-1} for the hollows. At this depth, rates of total C mineralisation in incubations of hollow and depression soils were significantly different to both each other and incubations of slope soils. Total rates of C mineralisation were significantly greater in incubations of soil from 0 - 5 than 5 - 15 cm in all cases. In incubations of soils from 0 - 5 cm the methanogenic fraction of total C mineralisation had means of 0.11 (0.01) for the upper slopes, 0.06 (0.01) for the lower slopes, 0.03 (NA) for the depression and 0.05 (0.01) for the hollows. The methanogenic fractions of total C mineralisation was significantly greater in the incubations of upper slope than lower slope and hollow soils. Similarly, mean methanogenic fractions for incubations of soils between 5 - 15 cm were 0.27 (0.09) for the upper slopes, 0.15 (0.04) for the lower slopes, 0.15 (0.02) for the depressions and 0.09 (0.01) for the hollows. Methanogenic fractions for incubations of depression and hollow soils were significantly different at this depth. The methanogenic fraction of total C mineralisation was significantly greater in incubations of soils from 5 - 15 cm that 0 - 5 cm for the lower slopes and hollows.

4.5.5.2 Relationships between in vitro rates of CH_4 cycling

Reflecting variations in edaphic conditions of the slope and basin soils of the study area, soil chemical and physical properties such as gravimetric water content, porosity, C content, N content, C:N were significantly positively correlated across incubations. Across the incubations of soils from both 0 - 5 cm and 5 - 15 cm, observed CO_2 fluxes were significantly positively correlated with such metrics; for example, the r^2 for linear regressions between observed CO_2 flux and soil C content for incubations of soils from 0 - 5 and 5 - 15 cm were 0.77 (n = 24) and 0.72 (n = 24), respectively. Observed net CH_4 flux, irrespective of the exclusion of incubations with rates in excess of 4.38 µg CH_4-C g dry soil^{-1} d^{-1}, were not significantly correlated with observed CO_2 flux or soil parameters across incubations of soils from 0 - 5 cm. In contrast, observed net CH_4 flux across incubations of soils from 5 - 15 cm was significantly negatively correlated with observed CO_2 flux (r^2 = 0.78, n = 24).

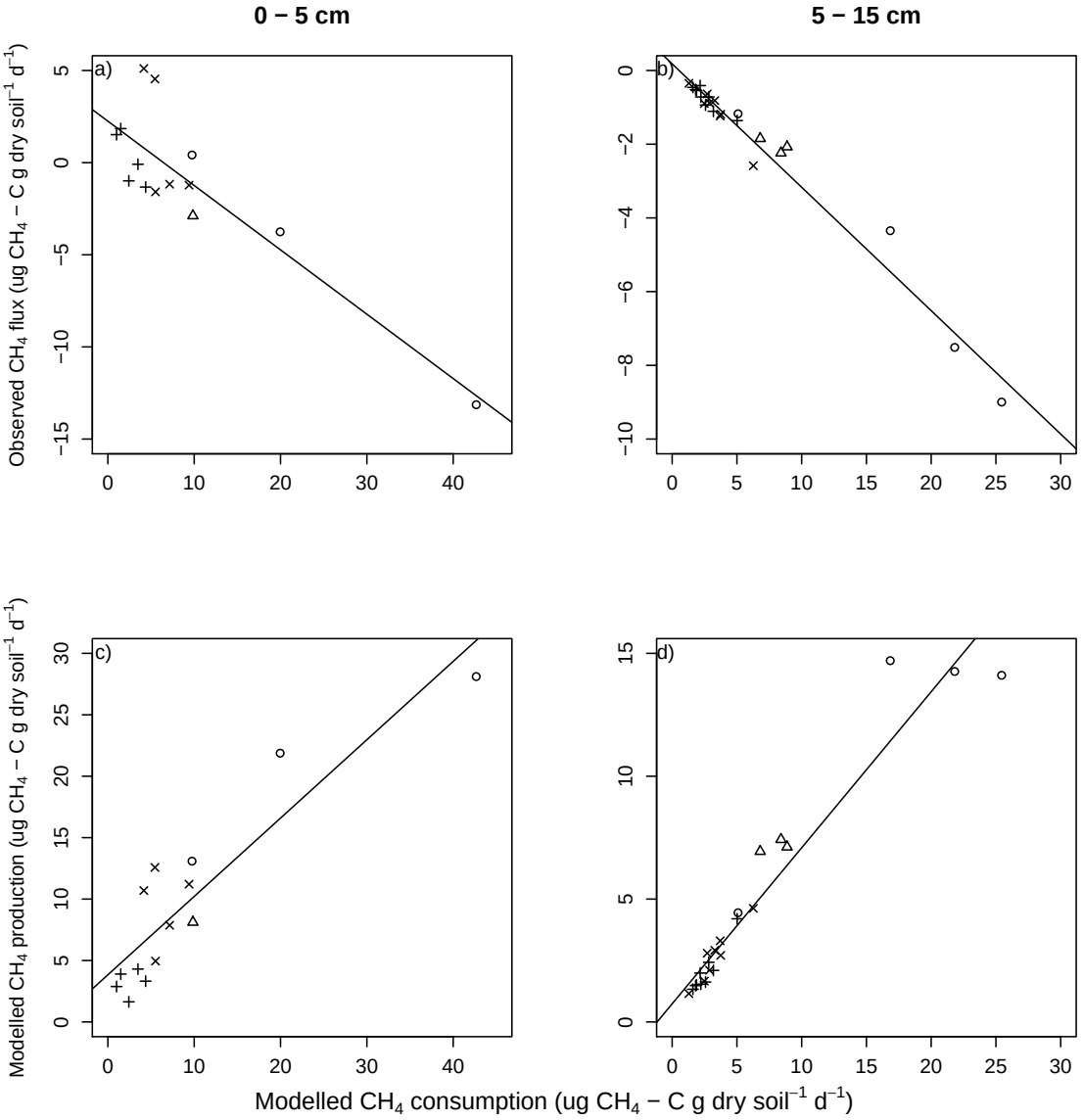

Figure 4.10: Relationships between observed in vitro net CH_4 flux and modelled gross rate of CH_4 consumption for soils from a) 0 - 5 cm (n = 14) and b) 5 - 15 cm (n = 24) and between modelled gross rate of CH_4 production and modelled gross rate of CH_4 consumption for soils from c) 0 - 5 cm (n = 14) and d) 5 - 15 cm (n = 24). Incubations of upper slope sampling station soils are indicated by ×, lower slope by + , depression by △ and hollows by ○.

Reflecting relationships between observed net CH_4 and CO_2 fluxes, observed net CH_4 flux was not significantly correlated with rate of total C mineralisation or the methanogenic fraction of C mineralisation across incubations of soils from 0 - 5 cm but was significantly negatively correlated with rate of total C mineralisation across incubations of soils from 5 - 15 cm (r^2 = 0.78, n = 24). However, observed net CH_4 flux was significantly negatively correlated with modelled rate of gross CH_4 consumption across incubations of soils from both 0 - 5 cm (r^2 = 0.74, n = 14) and 5 - 15 cm (r^2 = 0.97, n = 24) (Figure 4.10). In this context, modelled rates of CH_4 consumption and production were significantly positively correlated across incubations of soils from both 0 - 5 cm (r^2 = 0.81, n = 14) and 5 - 15 cm (r^2 = 0.93, n = 24) (Figure 4.10). In both cases these gross process rates were significantly positively correlated with rate of total C mineralisation but not the methanogenic fraction of C mineralisation. The r^2 for linear regressions between modelled rate of CH_4 consumption and total C mineralisation across incubations of soils from 0 - 5 cm and 5 - 15 cm are 0.46 (n = 14) and 0.87 (n = 24), respectively. Similarly, the modelled rate of CH_4 production and total C mineralisation across incubations of soils from 0 - 5 cm and 5 - 15 cm were significantly positively correlated with r^2 of 0.64 (n = 14) and 0.92 (n = 24), respectively.

4.6 Discussion

4.6.1 Humid puna landscapes as a source of atmospheric CH_4

Extension of the long-term monthly measurements of net CH_4 flux from a period of one to two and half years corroborates the previous finding that the soils of the study site are a source of atmospheric CH_4 (Teh et al., 2014). Similarly, we confirm the importance of seasonality for soil-atmosphere CH_4 exchange from such soils with plot mean net CH_4 fluxes ranging from -0.33 to 24.64 mg CH_4-C m^{-2} d^{-1} for dry season months and 0.11 to 181.74 mg CH_4-C m^{-2} d^{-1} for wet season months. These data support the indication that humid puna ecosystems are a regional hotspot for CH_4 emission when compared to sink activity reported for other Andean and western Amazonian upland environments. For example, Wolf et al. (2012) report CH_4 uptake rates of -1.62 to -

0.16 mg CH_4-C m^{-2} d^{-1} for montane and premontane forests in the Ecuadorian Andes. Similarly, Palm et al. (2002) indicate that upland soils under a variety of land-uses in the Peruvian Amazon principally act as a sink, with source activity only observed in sites of high-input cropping agriculture, for atmospheric CH_4 with net fluxes in the range of -0.72 to 0.36 mg CH_4-C m^{-2} d^{-1}.

Considerable spatial differences in soil-atmosphere CH_4 exchange are apparent among measurement plots for these long-term data suggesting that up scaling the activity of such landscapes to a regional basis will require appropriate land cover estimates at meso and micro topographical scales (Wania et al., 2009; McNamara et al., 2008; Teh et al., 2011, 2014). For example, emissions of CH_4 were dominated by the activity of the hollow plot within the basin of the study area where mean net fluxes for dry and wet season months were 24.65 (10.70) and 181.74 (36.45) mg CH_4-C m^{-2} d^{-1}. In contrast, the magnitude of soil-atmospere CH_4 exchange from the ridge, slope and depression plots were more marginal varying between uptake and emission among plot and between seasons with mean net CH_4 fluxes for dry and wet season months, respectively, of -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} on the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} on the slope and -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} in the depression. Contrasts between activity of the hollow and depression plots, where we might expect soil CH_4 cycling to reflect a wetland setting, may result from micro topographical differences in water table depth (Waddington and Roulet, 1996; Teh et al., 2011). However, emissions are also observed from the ridge and slope plots where topographic position and steep gradients suggest development of water table proximal to the surface is unlikely. Hence, traditional models of soil CH_4 cycling used to inventory sources and sinks of atmospheric CH_4 do not appear to be appropriate in describing mesoscale topographic variations in soil-atmosphere CH_4 exchange for this landscape (Dutaur and Verchot, 2007; Denman et al., 2007). In this respect, we find support for the growing body of evidence that suggest that wet upland soils in tropical highlands are likely an important but under represented source of atmospheric CH_4 (Silver et al., 1999; Megonigal and Guenther, 2008; Spahni et al., 2011). Notably, within plot increases in monthly mean of net CH_4 fluxes are typically associated with increased spatial variability between sampling stations. Indeed, instances of

contrasting uptake and emission of CH_4 by sampling stations within plots during the same sampling period are not uncommon. This behaviour indicates that constraints on soil-atmosphere CH_4 exchange are considerably variable at scales of tens of metres.

4.6.2 Landscape variability in soil-atmosphere CH_4 exchange

Intensive campaigns during wet and dry seasons were used to investigate the spatial patterns in net CH_4 flux among and within landscape features elucidated by the long-term measurements. These campaigns broadly support observations from the monthly plot measurements with mean net CH_4 fluxes from microform groups during dry and wet season campaigns, respectively, of -0.45 (0.12) and 0.22 (0.12) mg CH_4-C m^{-2} d^{-1} for the upper slopes, 1.43 (0.35) and 7.45 (1.31) mg CH_4-C m^{-2} d^{-1} for the lower slopes, -0.64 (0.06) and -0.23 (0.10) mg CH_4-C m^{-2} d^{-1} for the depressions and 16.26 (1.28) and 108.19 (10.32) mg CH_4-C m^{-2} d^{-1} for the hollows. Across the study site, mesoscale topographic variations were best delineated by slope angle and soil properties with organomineral soils found on the slopes whilst the basin contained peat soils indicative of wetland conditions (Clymo, 1984). Emissions from these upland and wetland soils are within ranges observed globally with mean rates of emissions from wet upland mineral soils reviewed by Spahni et al. (2011) ranging from 0.1 to 17.5 mg mg CH_4-C m^{-2} d^{-1} and from subtropical, temperate, boreal and subarctic wetlands reviewed by Turetsky et al. (2014) ranging from 36.2 to 84.2 mg CH_4-C m^{-2} d^{-1}.

Differences in soil-atmosphere CH_4 exchange from the microform groups on the slope or in the basin were not clearly differentiated by measured edaphic conditions such as variations in the composition and abundance of grasses which are important in determining soil-atmosphere exchange in many Northern hemisphere peatlands (Ström et al., 2003; Raghoebarsing et al., 2005; McNamara et al., 2008). For example, across the landscape variations in sampling station mean of wet and dry season campaign net CH_4 fluxes from marginal emission or uptake activity on the upper slopes and in the depressions to strong emission from the hollows was signigicantly correlated with metrics such as the above ground biomass of *Calamagrotis spp.* (Spearman's ρ = -0.56) and soil C:N (Spearman's ρ = 0.45), reflecting the transition from organomineral

to organic soils. However, for slope sampling stations, greater fluxes were related to shallower slope angles (Spearman's ρ = 0.53) at lower elevations and slight differences in the composition of sub-surface soils. Whilst across the basin differences were related to contrasts in particle density (Spearman's ρ = 0.79) at the surface and N content in the sub soils (Spearman's ρ = -0.82) reflecting the composition of peat soils in the hollows, comprised principally of moss litter, and more humidified material in the depressions. In this respect, we interpret these relationships as broadly reflecting hydrological conditions of the study area but do not infer direct influences of these metrics on soil-atmosphere CH_4 exchange based on our limited data (McNamara et al., 2008; Noyce et al., 2014; Turetsky et al., 2014) . We did not investigate the influence of plant mediated CH_4 transport in this study as grass tussocks are typically larger than the static flux chambers used. In this respect, the presence of aerenchymatous grasses indicate that vegetation may play an important role, with plant transport dominating soil-atmosphere CH_4 exchange of some wetland environments, in controlling net CH_4 fluxes from this landscape not captured by our measurements (Holzapfel-Pschorn and Seiler, 1986; Schimel, 1995; Shannon et al., 1996).

Notably, the portion of net CH_4 fluxes that were close to zero was high for some microforms (Verchot et al., 2000). For example, indeterminable fluxes during dry and wet season campaigns respectively accounted for 68 and 44 $\%$ of observations on the upper slopes and 30 and 26 $\%$ of observations in the depressions. Similarly, 43 $\%$ of net CH_4 flux observations were indeterminable on the lower slopes during the dry season campaign. Such observations, representing either weak emission or uptake, indicates that the methanogenic and methanotrophic processes controlling net CH_4 flux are finely balanced in these situations (Verchot et al., 2000; Von Fischer and Hedin, 2007). Thus, whilst the overall direction of soil-atmosphere CH_4 exchange reported here is reasonable there is less confidence in the magnitude, with true mean fluxes likely closer to zero, of reported mean net CH_4 fluxes from these features (Verchot et al., 2000).

4.6.3 Drivers of spatial variation in soil-atmosphere CH_4 exchange

In upland and wetland settings, variations in net CH_4 flux are most simplistically explained in terms of the influence of WFPS in upland soils and water table depth in wetland soils on the distribution of CH_4 and O_2 (Verchot et al., 2000; Turetsky et al., 2014). We find support for these relationships across the landscape with campaign means of sampling station net CH_4 flux significantly negatively correlated with water table depth (Spearman's ρ: wet season = -0.49, dry season = -0.57) and positively correlated with WFPS (Spearman's ρ: wet season = 0.47, dry season = 0.59) in both wet and dry season campaigns. Similarly, among basin sampling stations net CH_4 flux was significantly negatively correlated with water table depth (Spearman's ρ: wet season = -0.86, dry season = 0.93) and positively correlated with WFPS (Spearman's ρ: wet and dry season = 0.96). These relationships reflect the relative promotion of methanogenic over methanotrophic processes in the hollows, where surface soils are more saturated and water tables higher, compared to the depressions and slopes. In contrast, among slope sampling stations no significant relationships were identified between net CH_4 flux and WFPS or water table depth. Whilst evidence for water table development proximal to the surface in these soils is limited, a positive relationship with WFPS reflecting constraints on the diffusion of O_2 and CH_4 may be expected (Conrad, 1996; Verchot et al., 2000; Smith et al., 2003). The absence of such a relationship may reflect the fact that WFPS is high and relatively invariant, with dry and wet season means for the upper and lower slopes ranging from 78.1 to 84.2 $\%$, suggesting that these soils are persistently close to saturation.

We hypothesised that soil O_2 concentration, as the proximal constraint on the balance between methanogenic and methanotrophic activity to which WFPS and water table depth are proxy would best explain spatial variations in net CH_4 flux (Silver et al., 1999; Verchot et al., 2000; Turetsky et al., 2014). Generally we accept our hypothesis, with stronger negative correlations between net CH_4 flux and soil O_2 concentration (Spearman's ρ: wet season = -0.84, dry season = -0.81) across the landscape in both wet and dry season campaigns than observed for water table depth or WFPS. Notably, this relationship between net CH_4 flux and soil O_2 concentration held across the slope

sampling stations (Spearman's ρ: wet season = -0.75, dry season = -0.81). In this respect, we find agreement with previous studies that indicate measurements of soil O_2 concentration better characterise soil-atmosphere exchange than interpretation of hydrological proxies in wet upland soils with significant methanogenic activity (Silver et al., 1999; Schuur et al., 2001; Teh et al., 2005). Deviation from our hypothesis is seen across the basin where a significant negative correlation between net CH_4 flux and soil O_2 was identified for the dry season (Spearman's ρ = -0.96) but not the wet season campaign. This situation may arise as the interface between zones of oxic and anoxic conditions, reflected by a wet season campaign mean water table depth of 4 (1) cm, is close to the soil surface in the hollows. Soil O_2 concentration measurements, centered at 10 cm depth, below this interface may poorly characterise the balance between processes determining soil-atmosphere CH_4 exchange.

4.6.4 Drivers of spatial variation in below-ground CH_4 cycling

Observation of both uptake and emission of CH_4 across the landscape indicates that both methanogenic and high-affinity methanotrophic processes play a role in driving soil-atmosphere exchange (Von Fischer and Hedin, 2007). Soil CH_4 concentrations were in excess of atmospheric concentration in both dry and wet season campaigns. However, despite positive gradients in soil to atmosphere CH_4 concentration, sampling stations acting to uptake CH_4 are present in both the campaigns of both seasons. Such a situation indicates that the potential of low-affinity methanotrophy to track production and attenuate emission in these soils may be high (Von Fischer and Hedin, 2007; Hornibrook et al., 2009). Campaign sampling station mean net CH_4 fluxes and soil CH_4 concentration were significantly positively correlated across the landscape (Spearman's ρ = 0.79) and slope (Spearman's ρ = 0.67) in the wet season but not dry season. A positive relationship between net flux and soil CH_4 concentration support the inference that decreases in soil O_2 concentration increase net CH_4 fluxes by relatively promoting methanogenic over methanotrophic activity. However, the lack of relationships for basins and dry season slopes suggests that either consumption may be controlling fluxes or that the zone of production driving variations between uptake

and emission is poorly characterised, similarly to the O_2 concentration in the basins, by measurements at 10 cm in these situations.

In vitro incubation of surface soils indicate that hotspots of methane emission were present in the 0 - 5 cm layer across the landscape irrespective of oxic conditions. Whilst we found no significant relationships with this activity it may reflect the presence of hotspots of labile C, fueling methanogenic activity, in these surficial soils (Wachinger et al., 2000). Such activity may help explain the relationship between dry season slope net fluxes and soil CH_4 concentration. In contrast, net emissions were not observed in incubations of soils from 5 - 15 cm and net uptake rates were positively correlated with metrics, reflecting the transition from organomineral to peat soils, such as net CO_2 production (r^2 = 0.78). In this respect, the capacity for CH_4 uptake may be promoted in soils with higher organic contents, most notably in the hollow soils, through greater availability of ecological niches for methanotrophs in higher porosity soils, syntrophic relationships between methanotrophs and mosses or greater availability of non-CH_4 substrates that may support methanotrophs (Smith et al., 2003; Von Fischer et al., 2009; Dedysh et al., 2005; Kip et al., 2010).

Unfortunately, we were not able to deconvolve gross process rates for many of the net emission observations in incubations of soils from 0 - 5 cm (Figure 4.10). However, for soils with lower net flux rates, we find support for previous observations that methanogenic activity is widespread in oxic soils (Silver et al., 1999; Von Fischer and Hedin, 2002; Megonigal and Guenther, 2008). Similarly, we suggest that the capacity for consumption to track variations in rates of production is high (Von Fischer and Hedin, 2007; Hornibrook et al., 2009). Rates of modelled gross production and consumption were both significantly related to the rate of total C mineralisation. That is, greater rates of production were driven by general increases in the availability of C and that methanotrophic potentials in these soils were mostly greater than this activity under oxic conditions. Notably, the indication that production is more strongly related to total rather than methanogenic fraction of C mineralisation suggests that substrate competitions observed in wet tropical forest soils may not represent a significant control on methanogenesis across this landscape (Von Fischer and Hedin, 2007; Teh et al., 2008a;

Dubinsky et al., 2010). In this respect, the availability of C likely represents a control on the magnitude of field soil-atmosphere CH_4 exchange in response to variations in the extent of anoxic and oxic conditions. A better understanding of the O_2 and temperature sensitivity of methanogenesis and methanotrophy in these soils is likely required considering both anoxia and soil temperature are greatest during the wet season in this environment (Segers, 1998; Von Fischer and Hedin, 2007).

4.7 Conclusions

Here we report soil-atmosphere CH_4 exchange and associated environmental variables from a humid puna ecosystem in the Southeastern Andes of Peru. We specifically focused on the spatial variability and drivers of net CH_4 flux both across and within landscape features typical of such environments through field measurement campaigns and laboratory incubations of soils. The findings of this study support humid puna ecosystem as a regionally significant source of CH_4, most notably during the wet season when soil anoxia and temperature are greater, when compared to sink activity previously reported for Andean and Western Amazonian upland forests. Further work is required to constrain variability of soil-atmosphere CH_4 exchange in similar paramo and xeric puna ecosystems associated with climatic variability along and across the Andes. We note that whilst source activity is associated with both wetland, particularly from persistently wet hollow features, and upland soils there is considerable variability across the landscape of our study site. As such, upscaling soil-atmosphere CH_4 exchange from such environments will require estimates of land cover at both meso and micro topographical scales. Furthermore, we report fluxes from a single study area and further work is required to generalise the activity of such landscapes in terms of complex interactions between orography, precipitation and drainage in the Andes. We indicate that spatial patterns in soil-atmosphere CH_4 exchange, similarly to tropical montane forest sources of CH_4 in Puerto Rico, are best explained by soil O_2 concentration reflecting the relative balance between methanogenic and methanotrophic processes in upland soils. However, traditional metrics such as water table depth may be more sensitive in wetland settings. We find that soil CH_4 concentrations are in excess of atmospheric

levels across our study site in both dry and wet seasons indicating that low-affinity methanotrophic activity plays a significant role in limiting emissions and enables uptake of atmospheric CH_4 in some locations. Although not considered in this study, the presence of positive soil-atmosphere CH_4 gradients and aerenchymatous grasses suggests that source activity, through plant mediated transport, is larger than reported. Unsurprisingly, laboratory incubations indicate that methanogenic activity is ubiquitous in these soils, with hotspots of emission present in surficial soils, irrespective of oxic conditions. Deconvolution of gross methanogenic and methanotrophic processes in these incubations support the conclusion that methanotrophic potentials are high and capable of consuming significant quantities of endogenous CH_4. Whilst rates of gross CH_4 production are driven by general increases in the availability of soil C to microbial communities. In this respect, a better understanding of the interaction between O_2 and temperature sensitivity of methanogenic and methanotrophic processes in these soils is likely to be key in understanding the magnitude of both spatial and temporal variations in soil-atmosphere CH_4 exchange from such soils.

Chapter 5

General discussion

The results chapters of this thesis are presented in a journal format to address pertinent questions relating to variability in soil-atmosphere CH_4 exchange and soil CH_4 cycling in a variety of poorly studied, upland soils of the Peruvian Andes and Amazon. In Chapter 2 we focused on differences in the drivers of net CH_4 flux and the distribution of methanogenic and methanotrophic processes within two lowland terra firme rainforest soils. In Chapter 3 we focused on variations and drivers of net CH_4 flux among and within premontane, lower montane and upper montane forests. In Chapter 4 we focused on drivers of net CH_4 flux and relationships between methanogenic and methanotrophic processes across the upland and wetland soils of a montane humid puna grassland. Here we synthesise the findings of these chapters in terms of three hypotheses that underpinned this work; namely, the expectation that montane upland soils would represent a source or weaker sink of atmospheric CH_4 than their lowland counterparts, that variations in soil O_2 concentration would drive net CH_4 fluxes in these environments and that methanogenic processes would play a role in soil CH_4 cycling within these soils despite oxic conditions.

5.1 Sources and sinks of atmospheric CH_4 from upland and montane soils

In contrast to polar and temperate latitudes, discrepancies between top-down and bottom-up estimates of the tropical atmospheric CH_4 budget suggests that current source-sink inventories under estimate the magnitude of emissions to the atmosphere from regions such as tropical South America (Mikaloff Fletcher et al., 2004b; Frankenberg et al., 2008; Bloom et al., 2010b). Similarly, unattributed sources of atmospheric CH_4 have been identified within tropical South American landscapes (Melack et al., 2004; do Carmo et al., 2006; Miller et al., 2007; Querino et al., 2011). Such a situation indicates that variability in soil-atmosphere exchange for known sources and sinks of atmospheric CH_4 and associated estimates of their extent used in scaling these activities is poorly constrained. Improvements in these respects are required as tropical South America constitutes a significant component of the global CH_4 cycle and has been implicated in playing an important role in driving Quaternary variability in atmospheric CH_4 concentration at multiple time scales (Mikaloff Fletcher et al., 2004b; Frankenberg et al., 2008; Bousquet et al., 2006; Singarayer et al., 2011). Consequently, better constraints on the role of soils, which act as both significant sources and sinks for atmospheric CH_4, are required (Mikaloff Fletcher et al., 2004b; Frankenberg et al., 2008; Dutaur and Verchot, 2007; Bloom et al., 2010b).

Estimates of soil-atmosphere CH_4 exchange in tropical South America have focused on activity of lowland environments in the Amazon basin with sinks associated with uptake in the upland soils of extensive terra firme rainforests (Potter et al., 1996; Dutaur and Verchot, 2007) and sources associated with emissions from wetlands (Melack et al., 2004; Ringeval et al., 2010; Bloom et al., 2012). However, observations from these forested upland soils (Keller et al., 1986; Steudler et al., 1996; Verchot et al., 2000; Palm et al., 2002; Fernandes et al., 2002; Keller et al., 2005; Davidson et al., 2004, 2008; Neto et al., 2011) and wetland swamp forests, seasonally inundated floodplain forests, rivers and lakes (Richey et al., 1988; Devol et al., 1988, 1990; Bartlett et al., 1990; Engle and Melack, 2000; Smith et al., 2000b; Lima, 2005; Marani and

Alvala, 2007; Bastviken et al., 2010; Belger et al., 2011; Sawakuchi et al., 2014) are relatively limited, reflecting geographic research biases and difficulty of access, when compared to a long history of such research in the Northern Hemisphere (Potter et al., 1996; Dutaur and Verchot, 2007; Turetsky et al., 2014; Sjögersten et al., 2014). Notably, there is a growing body of evidence to suggest that under certain conditions upland soils can emit CH_4 and that such activity potentially represents a globally significant source to the atmosphere (Spahni et al., 2011). Indeed, whilst Amazonian forested upland soils predominately act to uptake atmospheric CH_4, sporadic instances of CH_4 emission are commonly identified within these environments (Keller et al., 1986; Davidson et al., 2004; Verchot et al., 2000). Such activity may be particularly pertinent in humid montane regions where the combination of high precipitation, temperatures and soil C stocks may favour source activity (Silver et al., 1999; Teh et al., 2005). Despite the extensive nature of the tropical Andes, elevations above 500 to 600 m asl have not typically been considered in regional budget estimates and there are very few observations of soil-atmosphere CH_4 exchange from montane upland soils in South America (Neto et al., 2011; Wolf et al., 2012; Teh et al., 2014). Similarly, the presence of high altitude wetlands within the upland settings of Andean paramo and puna is well documented (Junk, 1993; Josse et al., 2009a; Earle et al., 2003; Squeo et al., 2006; Pansu et al., 2007; Otto et al., 2011; Höfle et al., 2013; Carilla et al., 2013; Segnini et al., 2013). However, published accounts of soil-atmosphere CH_4 exchange are notably lacking for such environments (Fritz et al., 2011; Teh et al., 2014).

The previous chapters of this thesis documented soil-atmosphere CH_4 exchange from predominately upland soil settings on the eastern slope of the tropical Andes and western Amazon of Peru. A principal motivation of this work was to address the paucity of information relating to the direction and magnitude of soil-atmosphere CH_4 exchange in the lowland terra firme rainforests (Palm et al., 2002), tropical montane forests (Wolf et al., 2012; Teh et al., 2014) and montane grasslands and wetlands (Teh et al., 2014) of this region. We hypothesised that montane upland soils would represent a source or weaker sink of CH_4 to the atmosphere, which may aid in accounting for budgetary discrepancies, than their lowland counterparts.

In Chapter 2, we report soil-atmosphere CH_4 exchange, based on intensive seasonal campaigns, for two Western Amazonian terra firme rainforest sites growing on upland ultisol and and inceptisol soils. These soils principally acted a sink for atmospheric CH_4 with dry and wet season campaign mean net CH_4 fluxes, respectively, of -1.56 (0.06) and -1.39 (0.07) mg CH_4-C m^{-2} d^{-1} for the ultisol and -0.95 (0.06) and -0.41 (0.10) mg CH_4-C m^{-2} d^{-1} for the inceptisol. In this sense, soil-atmosphere exchange in these sites is comparable to that of other terra firme Amazonian forests where previously reported mean net CH_4 fluxes range from -1.35 to -0.06 mg CH_4-C m^{-2} d^{-1} (Keller et al., 1986; Steudler et al., 1996; Palm et al., 2002; Fernandes et al., 2002; Verchot et al., 2000; Keller et al., 2005; Davidson et al., 2004, 2008; Neto et al., 2011) (Table 5.1). Rates of CH_4 uptake in the ultisol soils are high compared to those reported for lowland forests but slower than that reported for an Ecuadorian premontane forest, with a mean of -1.62 mg CH_4-C m^{-2} d^{-1}, situated in the Northern Andes (Wolf et al., 2012).

In Chapter 3, we report soil-atmosphere CH_4 exchange, based on monthly measurements between January 2011 and June 2013 for a premontane forest, lower montane cloud forest and upper montane cloud forest site. Similarly to the lowland forests, these soils were predominately a sink for atmospheric CH_4 with means for aggregated dry and wet seasons net CH_4 flux, respectively, of -0.20 (0.15) and -0.08 (0.13) for the premontane forest, -1.12 (0.13) and, -0.97 (0.11) for the lower montane cloud forest and -1.55 (0.13) and -1.04 (0.11) for the upper montane cloud forest. Globally mean net CH_4 fluxes reported for tropical forest soils above 600 m asl range from -1.62 to -0.02 mg CH_4-C m^{-2} d^{-1} (Delmas et al., 1992; Ishizuka et al., 2005a; Purbopuspito et al., 2006; Werner et al., 2006; Kiese et al., 2008; Neto et al., 2011; Wolf et al., 2012). Notably, we identify contrasting patterns in the strength of CH_4 uptake with elevation when compared to similar studies in Ecuador and Indonesia (Purbopuspito et al., 2006; Wolf et al., 2012). Uptake was positively correlated with elevation in Peru, negatively correlated with elevation in Ecuador and no trend with elevation was identified in Indonesia. In this sense, we do not find evidence for the strong source activity identified in Puerto Rican tropical montane forests and instead indicate that such Andean forest soils play a broadly similar role to analogous upland soil environments both in the

Table 5.1: Mean CH_4 fluxes reported in this thesis compared with those previously reported for a variety of environments. References: 1 - Steudler et al. (1996); Fernandes et al. (2002); Keller et al. (2005), 2 - Keller et al. (1986); Verchot et al. (2000); Keller et al. (2005); Davidson et al. (2008), 3 -Delmas et al. (1992); Purbopuspito et al. (2006); Kiese et al. (2008); Neto et al. (2011); Wolf et al. (2012), 4 -Purbopuspito et al. (2006); Wolf et al. (2012), 5 -Purbopuspito et al. (2006); Wolf et al. (2012), 6 - Dutaur and Verchot (2007) and references therein, 7 - Spahni et al. (2011) and references therein, 8 - Turetsky et al. (2014), 9 - Richey et al. (1988); Devol et al. (1988, 1990); Bartlett et al. (1990); Engle and Melack (2000); Smith et al. (2000b); Lima (2005); Marani and Alvala (2007); Bastviken et al. (2010); Belger et al. (2011); Turetsky et al. (2014); Sawakuchi et al. (2014).

Site	Measurement	Mean CH_4 flux	Chapter	Environment	Mean CH_4 flux range	References
-	scale	(mg CH_4-C m^{-2} d^{-1})	this study	-	(mg CH_4-C m^{-2} d^{-1})	-
Lowland ultisol	seasonal	-1.49 (0.05)	Ch.2	Lowland ultisol	-1.22 to -0.20	1
Lowland inceptisol	seasonal	-0.68 (0.06)	Ch.2	Lowland oxisol	-0.49 to -0.06	2
Premontane forest	monthly	-0.14 (0.10)	Ch.3	Premontane forest	-1.55 to -0.20	3
Lower montane forest	monthly	-1.05 (0.09)	Ch.3	Lower montane forest	-0.91 to -0.85	4
Upper montane forest	monthly	-1.30 (0.09)	Ch.3	Upper montane forest	-0.40 to -0.29	5
Puna upland	monthly	1.12 (0.22)	Ch.4	Upland sinks	- 5.7 to 0.0	6
“	seasonal	2.16 (0.34)	Ch.4	Upland sources	0.0 to 17.5	7
Puna wetland	monthly	51.57 (9.47)	Ch.4	Northern wetlands	54.5 to 84.2	8
”	seasonal	30.90 (2.60)	Ch.4	Tropical wetlands	-4.8 to 442.5	9

lowland Amazon and tropical montane settings globally (Silver et al., 1999; Teh et al., 2005) (Table 5.1).

Finally, in Chapter 4 we report soil-atmosphere CH_4 exchange, based on monthly measurements between January 2011 and June 2013 and intensive seasonal campaigns, from upland and wetland soils from a montane humid puna grassland. Source-sink activity in this environment was variable with means for aggregated dry and wet season net CH_4 flux, respectively, of -0.33 (0.30) and 1.30 (0.56) for upland ridges, 0.64 (0.16) and 2.88 (0.60) for upland slopes, -0.30 (0.18) and 0.11 (0.27) for wetland depressions and 24.74 (10.70) and 181.74 (36.35) for wetland hollows. Similarly, means of dry and wet season campaign net CH_4 fluxes were, respectively, -0.45 (0.12) and 0.22 (0.12) mg CH_4-C m^{-2} d^{-1} for upland upper slopes, 1.43 (0.35) and 7.45 (1.31) mg CH_4-C m^{-2} d^{-1} for upland lower slopes, -0.64 (0.06) and -0.23 (0.10) mg CH_4-C m^{-2} d^{-1} for wetland depressions and 16.26 (1.28) and 108.19 (10.32) mg CH_4-C m^{-2} d^{-1} for wetland hollows. Rates of emission from these soils are comparable to those reviewed for upland soils ranging from 0.1 to 17.5 mg mg CH_4-C m^{-2} d^{-1}, and from subtropical, temperate, boreal and subarctic wetlands ranging from 36.2 to 84.2 mg CH_4-C m^{-2} d^{-1} (Spahni et al., 2011; Turetsky et al., 2014) (Table 5.1). We suggest that both the upland and wetland soils of such environments represent a previously undocumented but potentially significant regional source of atmospheric CH_4 when compared to the previously discussed patterns in uptake activity across proximal and more widespread upland forests (Fritz et al., 2011; Teh et al., 2014). Indeed, soil-atmosphere CH_4 exchange rates from our study site fall within the range, -4.8 to 442.5 mg CH_4-C m^{-2} d^{-1}, reported for wetland features in lowland Amazonian (Richey et al., 1988; Devol et al., 1988, 1990; Bartlett et al., 1990; Engle and Melack, 2000; Smith et al., 2000b; Lima, 2005; Marani and Alvala, 2007; Bastviken et al., 2010; Belger et al., 2011; Sawakuchi et al., 2014).

Hence we found partial support for our hypothesis that net CH_4 fluxes from montane upland soils would be more positive than from their lowland counterparts. Uptake rates for both the lowland and montane forest soils reported here are within previously reported ranges. In contrast we identified potentially significant source activity from

both the upland and wetland soils of the montane grassland. Considering the paucity of observations and the limited resolution of Andean land cover maps, up-scaling estimates of soil-atmosphere CH_4 exchange to landscape scales is a tenuous exercise (Josse et al., 2009b; Wolf et al., 2012; Teh et al., 2014). Nevertheless, the Kosnipata Valley, where our montane measurements were focused, is estimated to cover 30180 km^2 between 600 and 3700 m asl (Feeley and Silman, 2010; Teh et al., 2014). A simple area weighted estimate of soil-atmosphere CH_4 exchange for the study area can be made using the gross simplification that land cover is dominated by premontane forest between 600 and 1200 m asl, lower montane forest between 1200 and 2200 m asl, upper montane forest between 2200 and 3200 m asl and montane grassland between 3200 and 3700 m asl (Feeley and Silman, 2010; Teh et al., 2014). Surface area between 600 and 1200 m asl, 1200 and 2200 m asl, 2200 and 3200 m asl and 3200 and 3700 m asl respectively accounts for 24.30 $\%$, 29.56 $\%$, 26.72 $\%$ and 19.41 $\%$ of the total area (Feeley and Silman, 2010; Teh et al., 2014). Estimates of the proportional extent of wetland features within Andean montane grasslands are limited, however, Otto et al. (2011) suggest that wetlands may account for ~ 6 $\%$ of land cover across the xeric puna of southern Peru. Adopting this value yields estimates for upland and wetland settings, within the 3200 and 3700 m asl band, accounting for 18.25 $\%$ and 1.16 $\%$ of total area for the region. For the forest sites, mean net CH_4 flux rates were applied as reported from the long-term measurements. Similarly, long-term measurement means were used in the montane grassland with simple averages for upland soils calculated from the ridge and slope plots and for wetland soils from the depression and hollow plots . Whilst simplistic, this approach indicates that on balance the upland soils of the Kosnipata area act as a net sink for atmospheric CH_4 in both the dry and wet season (Table 5.2). However, the activity of wetland features, despite their small areal extent, is sufficient to drive the landscape from a net sink during the dry season to a net source of atmospheric CH_4 during the wet season. In this sense, we find support for the relevance of considering montane environments in revisions of South American CH_4 budgets.

Table 5.2: Area weighted seasonal means of soil-atmosphere CH_4 fluxes from long-term measurements in premontane, lower and upper montane forests and humid puna grasslands for the Kosnipata reigion, Peru.

Environment -	Elevation band (m asl)	Area (km^2)	Area (fractional)	*dry season* Mean CH_4 flux (mg CH_4-C m^{-2} d^{-1})	*wet season* Mean CH_4 flux (mg CH_4-C m^{-2} d^{-1})	*dry season* Weighted CH_4 flux (mg CH_4-C m^{-2} d^{-1})	*wet season* Weighted CH_4 flux (mg CH_4-C m^{-2} d^{-1})
Premontane	600-1200	7334	0.24	-0.20 (0.15)	-0.08 (0.13)	-0.05 (0.04)	-0.02 (0.03)
Lower montane	1200-2200	8923	0.30	-1.12 (0.13)	-0.97 (0.11)	-0.33 (0.04)	-0.29 (0.03)
Upper montane	2200-3200	8065	0.27	-1.55 (0.13)	-1.04 (0.11)	-0.41 (0.03)	-0.28 (0.03)
Upland puna	3200-3700	5507	0.18	0.16 (0.17)	2.11 (0.41)	0.03 (0.03)	0.39 (0.07)
Wetland puna	3200-3700	351	0.01	12.22 (5.35)	90.93 (18.18)	0.14 (0.06)	1.06 (0.21)
	Total	30180	1.00			-0.62 (0.09)	0.86 (0.23)

5.2 Drivers of soil-atmosphere CH_4 exchange

Sources and sinks of atmospheric CH_4 are respectively associated with wetland and upland soils owing to the different O_2 requirements of methanogenic and methanotrophic microbial communities driving the production and consumption of CH_4 (Conrad, 1996). In wetlands, variations in soil-atmosphere CH_4 exchange, modulated by transport processes, vegetation and temperature, are broadly driven by variations in water table depth through control on the relative extent of anoxic and oxic conditions (Turetsky et al., 2014). In well aerated upland soils, variations in soil-atmosphere CH_4 exchange are driven by the influence of soil texture and water content, typically reflected by measures of WFPS, on the diffusion supply of atmospheric CH_4 to high-affinity methanotrophs (Bender and Conrad, 1992, 1994; Teh et al., 2006). As previously discussed, observations of CH_4 emissions from upland soils are not uncommon and indicates that methanogenesis may also play a role in determining soil-atmosphere CH_4 exchange in these soils. Emissions of CH_4 in such situations have been described in terms of hotspot activity and the influence of WFPS and aerobic respiration, through diffusional constraints and in situ depletion, on the extent of anoxic microsites (Verchot et al., 2000; Davidson et al., 2004; Spahni et al., 2011). The importance of soil O_2 in tropical upland soils has been exemplified in the Luquillo Experimental Forest, Puerto Rico, where transition from CH_4 uptake to emission between lowland and montane forests was driven by decreases in O_2 concentration within the bulk soil atmosphere (Silver et al., 1999; Teh et al., 2005). Despite this, such measurements are generally lacking in studies of CH_4 cycling within tropical South American upland soils.

In tandem with our previously discussed aim to document soil-atmosphere CH_4 exchange, we also investigated the environmental drivers of variability in net CH_4 fluxes within the studied lowland terra firme rainforest (Chapter 2), tropical montane forest (Chapter 3) and montane puna grassland (Chapter 4) sites. We hypothesised that soil O_2 concentration, as an integrated measure of the influence of diffusional constraints and in situ biological demand on the presence of anoxic microsites, would best explain variations in net CH_4 flux in these studies.

In the lowland and montane forest studies, soil-atmosphere CH_4 exchange was dominated by uptake from the atmosphere and the soils were principally oxic with seasonal site means for net CH_4 flux and soil O_2 concentration at 10 cm ranging from -1.55 (0.06) to -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} and 17.9 (0.1) to 20.8 (0.1) $\%$. We found that variations between the ultisol and inceptisol soils in the lowland forests and among the premontane, lower montane and upper montane forests were best explained by a positive relationship with WFPS and a weaker negative relationship with CO_2 flux. As we find no evidence for significant anoxia, we interpret the observed increases in net CH_4 flux at higher WFPS in terms of diffusional constraints on the supply of atmospheric CH_4 to high-affinity methanotrophic communities (Bender and Conrad, 1992, 1994; Verchot et al., 2000; Teh et al., 2006; Curry, 2007). The weaker effect associated with CO_2 flux is unclear but may indicate that conditions preferable to general microbial activity also promote methanotrophy or that at higher WFPS anoxia, not effectively captured in our measurements, is limiting aerobic respiration and promoting low levels of methanogenic activity (Schuur et al., 2001; Wolf et al., 2012). Drivers of net CH_4 flux were not consistent within forest sites, for example no significant relationships were found with measured environmental variables within the lowland ultisol or the premontane forests, whereas, positive relationships with WFPS and negative relationships with CO_2 flux best explained variability within the lowland inceptisol and upper montane forests. Such differences may reflect local edaphic conditions with greater temporal variability in response to seasonal rainfall observed in the lowland inceptisol and upper montane forests. Considering contrasts in soil-atmosphere CH_4 exchange in these sites, with both the lowland inceptisol and premontane forest soils exhibiting sporadic emissions of CH_4 which were not prominent in the lowland ultisol or upper montane forest, this suggests that local biophysical constraints on CH_4 cycling are important in constraining soil-atmosphere exchange (Von Fischer et al., 2009; Hall et al., 2013).

In contrast to the forest studies, soil-atmosphere CH_4 exchange in the montane puna grassland varied between uptake and emission for both upland and wetland soils. In this environment variations in soil O_2 concentration, with seasonal microform means ranging from 20.1 (0.1) to 0.2 (0.2) $\%$ at 10 cm, provided a better explanation for

variations in net CH_4 fluxes across the upland soils and the landscape as a whole than WFPS or water table depth. However, this relationship broke down across the wetland soils during the wet season when the water table approached the surface, creating fully anoxic conditions at 10 cm below the soil surface, in some sites. Therefore, we suggest that negative correlation between soil O_2 concentration and net CH_4 flux reflects variations in the relative balance between production and consumption of CH_4 either through promotion of methanogenesis or suppression of methanotrophy as O_2 concentrations decrease (Silver et al., 1999; Teh et al., 2005). The upland soils appear to maintain principally oxic bulk soil atmospheres with seasonal microform means ranging from 12.5 (0.6) to 20.1 (0.1) $\%$. Considering that methanotrophic activity appears to be relatively insensitive to variations in O_2 concentration in excess of 3 $\%$ this may indicate that the identified relationship is driven by the ability of methanogenic communities to exploit expansion of anoxic conditions (Bender and Conrad, 1994; Teh et al., 2005, 2006; Von Fischer and Hedin, 2007).

Overall we found partial support for our hypothesis, reflecting dominant patterns in soil-atmosphere exchange, that variations in net CH_4 fluxes would be driven by soil O_2 concentration. In the forest environment, which principally acted to uptake atmospheric CH_4 we reject our hypothesis as the influence of soil texture and water content, reflected in measures of WFPS, best explained variations in net fluxes. Such observations conform to well documented expectations relating the activity of high-affinity methanotrophic communities to the diffusional supply of atmospheric CH_4 (Bender and Conrad, 1992; Verchot et al., 2000; Smith et al., 2003). In contrast, we accept our hypothesis in the montane puna grassland where variations in soil O_2 concentration best explained variations from net uptake to net emission across the landscape (Silver et al., 1999; Teh et al., 2005). We suggest that this relationship is underpinned by the response of methanogenic activity to changes in O_2 concentration. Across the transect, patterns between net CH_4 flux and WFPS or soil O_2 are not clear (Table 5.3). In this sense, understanding local biophysical constraints such as water stresses, non-linear constraints on supply of substrates to methanotrophic and methanogenic processes and the physical distribution of the microbial communities involved in these processes are likely important in constraining the source and sink activity of such tropical upland

soils (Von Fischer and Hedin, 2007; Davidson et al., 2008; Von Fischer et al., 2009; Dubinsky et al., 2010; Hall et al., 2013).

5.3 Relationships between methanogenesis and methanotrophy

Soil-atmosphere CH_4 exchange fundamentally results from the local balance between methanogenic production and methanotrophic consumption of CH_4 within the soil profile (Le Mer and Roger, 2001). Compensation concepts have been used to describe the transition between emission and uptake of gases such as nitric oxide in upland soils where production and consumption processes driving soil-atmosphere exchange are proximal to each other (Conrad, 1996). That is to say, the net flux rate at the surface is the production rate minus the product of the first order constant for consumption and the concentration of the species in the environment (Conrad, 1996; Von Fischer and Hedin, 2007). Whilst this model can be applied to CH_4 cycling in closed systems, it is less applicable to variations between uptake and emission in field measurements where methanogenic and methanotrophic processes may be spatially stratified (Conrad, 1996; Von Fischer and Hedin, 2007). For example, in wetland soils methanogenic maxima are located at depth below the water table whilst the activity of low-affinity methanotrophy peaks close to the interface between oxic and anoxic zones (Sundh et al., 1994; Amaral and Knowles, 1995; Conrad, 1996; Hornibrook et al., 2009). In such settings, soil-atmosphere CH_4 exchange is driven by concentration gradients between the zone of production and atmosphere modulated by the efficiency of intervening methanotrophic zones (Conrad, 1996; Hornibrook et al., 2009). The spatial distribution of methanogenic and methanotrophic processes are less well defined in upland soils. Depth profiles of CH_4 concentration and soil incubations typically indicate that high-affinity methanotrophic activity is maximal in superficial mineral soils below organic horizons (Adamsen and King, 1993; Bender and Conrad, 1994; Priemé and Christensen, 1997; Hütsch, 1998; Verchot et al., 2000; Davidson et al., 2004; Sjögersten et al., 2007; Wolf et al., 2012). This preference presumably relates to the ecological requirements of such communities involving the diffusional supply of atmospheric

Table 5.3: Means and standard errors for net CH_4 fluxes, WFPS and soil O_2 concentrations at 10 cm depth reported in the previous chapters.

		dry season			*wet season*		
Site	Measurement	Mean CH_4 flux	WFPS	[O_2]	Mean CH_4 flux	WFPS	[O_2]
-	scale	(mg CH_4-C m^{-2} d^{-1})	(%)	(%)	(mg CH_4-C m^{-2} d^{-1})	(%)	(%)
Lowland ultisol	seasonal	-1.56 (0.06)	30.6 (0.5)	20.8 (0.1)	-1.39 (0.07)	43.2 (0.9)	17.9 (0.1)
Lowland inceptisol	seasonal	-0.95 (0.06)	39.6 (0.9)	20.5 (0.1)	-0.41 (0.10)	63.3 (0.9)	18.1 (0.1)
Premontane	monthly	-0.20 (0.15)	51.5 (2.1)	19.7 (0.3)	-0.08 (0.13)	53.4 (1.9)	19.8 (0.2)
Lower montane	monthly	-1.12 (0.13)	34.0 (2.0)	19.1 (0.3)	-0.97 (0.11)	42.3 (1.6)	19.2 (0.2)
Upper montane	monthly	-1.55 (0.13)	23.6 (2.0)	18.6 (0.3)	-1.04 (0.11)	41.8 (1.6)	18.8 (0.2)
Puna ridge	monthly	-0.33 (0.30)	NA	18.5 (0.6)	1.30 (0.56)	NA	16.4 (0.8)
Puna slope	monthly	0.64 (0.16)	NA	16.8 (1.0)	2.88 (0.60)	NA	13.1 (1.8)
Puna depression	monthly	-0.30 (0.18)	NA	16.1 (1.3)	0.11 (0.27)	NA	11.0 (1.4)
Puna hollow	monthly	24.74 (10.70)	NA	9.5 (3.3)	181.74 (36.35)	NA	5.3 (3.3)
Puna upper slope	seasonal	-0.45 (0.12)	84.2 (1.1)	20.0 (0.1)	0.22 (0.12)	81.4 (1.1)	16.8 (0.3)
Puna lower slope	seasonal	1.43 (0.35)	84.1 (1.1)	18.7 (0.3)	7.45 (1.31)	78.1 (1.0)	12.5 (0.6)
Puna depression	seasonal	-0.64 (0.06)	75.8 (1.7)	20.1 (0.1)	-0.23 (0.10)	80.4 (1.5)	16.9 (0.8)
Puna hollow	seasonal	16.26 (1.28)	96.0 (0.7)	16.6 (0.9)	108.19 (10.32)	91.7 (0.6)	0.2 (0.2)

CH_4, sensitivity to water stresses and interactions with nitrogen availability (Conrad, 1996; Reay and Nedwell, 2004; Von Fischer et al., 2009). Methanogenic maxima have been identified both in superficial organic and mineral horizons of upland soils (Sexstone and Mains, 1990; Yavitt et al., 1990; Teh et al., 2005; Wolf et al., 2012). In this sense, the relative position of zones of production and consumption and constraints on their individual activities has implications for conceptualising the relationship between environmental conditions and soil-atmosphere exchange (Conrad, 1996). In particular, emissions of CH_4 from broadly oxic upland soils, where we might not expect variations in O_2 concentrations to suppress methanotrophic activity, seem to be driven by variations in methanogenic rather than methanotrophic activity (Bender and Conrad, 1994; Silver et al., 1999; Teh et al., 2005, 2006; Von Fischer and Hedin, 2007).

In addition to our previously discussed aims to document soil-atmosphere CH_4 exchange and investigate the environmental drivers of this activity, we also investigated variability in below-ground CH_4 cycling and relationships between methanogenic and methanotrophic processes within the studied lowland terra firme rainforest (Chapter 2) and montane puna grassland (Chapter 4) sites. We hypothesised that methanogenic processes would be active, despite oxic conditions, in these soils and expected variations in soil CH_4 cycling to be driven by methanogenic activity.

Incubations of lowland forest soils from 0 - 5 cm, 5 - 15 cm, 25 - 35cm, 45 - 55 cm at wet season field conditions and under oxic headspaces principally acted to uptake CH_4 at close to atmospheric concentrations. This uptake activity was greatest at 0 - 5 cm in the inceptisol soils and 5 - 15 cm in the ultisols. The uptake of atmospheric CH_4 which dominated field measurements at these sites was likely driven by activity high-affinity methanotrophic communities in these superficial mineral soils. Deconvolution of gross methanotrophic and methanogenic process rates in these incubations using an isotope pool dilution technique indicated the despite oxic conditions background methanogenesis was occurring, presumably within anoxic microsites, throughout the profiles of both soils (Von Fischer and Hedin, 2002). Maxima in CH_4 consumption at 0 - 5 cm in the inceptisols and 5 - 15 cm in the ultisols appeared to be driven by increases in methanotrophic rather than decreases in methanogenic activity. Within

these incubations, we were not able to identify significant relationships, potentially reflecting the relatively small variations within and among these soils, between net flux, modelled gross methanotrophic and methanogenic process rates and measured physical and chemical soil parameters. However, it seems plausible that methanotrophic rates peaked in the superficial soils as a result of the positive influence of higher porosity on the diffusional supply of CH_4 and the sensitivity of microbial communities to variations in soil moisture (Smith et al., 2003; Von Fischer et al., 2009). Notably, during the wet season field campaign sporadic emissions of CH_4 were observed and soil CH_4 concentrations exceeded atmospheric levels in some localities within the inceptisol soil. We did not identify zones of emissions in incubations of these soils to support this activity, however, gross process rates were most similar in incubations of the inceptisols from 25 - 35 cm. In the field, soil CH_4 concentration was mostly strongly negatively correlated with soil O_2 and CO_2 concentration at 30 cm suggesting that the observed source activity may have been driven by variations in the balance between methanogenic and methanotrophic processes in these deeper soils (Teh et al., 2005; Liptzin et al., 2011).

In contrast, net CH_4 fluxes in incubations of puna soils from 0 - 5 cm and 5 - 15 cm at wet season field conditions and under oxic headspaces were varied. In incubations of soils from 0 - 5 cm both emission and uptake activity was observed, whilst, incubations of soils from 5 - 15 cm principally acted to uptake CH_4 at close to atmospheric concentrations. These incubations indicate that field observation of CH_4 emissions from upland soils in the puna may be driven by the relationship between variations in soil O_2 concentration and the activity of anoxic microsites close to the surface. We were unable to explain variations between uptake and emission in incubations of these surface soils using measured physical and chemical soil parameters and we were not able to deconvolve gross methanotrophic and methanogenic process rates for the majority of incubations exhibiting emissions (Wachinger et al., 2000). In contrast, in incubations of soils from 5 - 15 cm increases in rates of CH_4 uptake were driven by metrics associated with the transition from organomineral to peat soils potentially reflecting the positive influence of higher porosity on high-affinity methanotrophic activity (Smith et al., 2003; Von Fischer et al., 2009). Deconvolving gross processes within incuba-

tions that exhibited uptake of CH_4 indicated that methanogenic activity is ubiquitous in the soils of this environment. Uptake rates in these incubations were positively correlated with both gross rates of production and consumption, indicating a general capacity for methanotrophy to track methanogenesis that may explain observations of CH_4 uptake in the field despite the presence of soil CH_4 concentrations in excess of atmospheric levels (Von Fischer and Hedin, 2007; Hornibrook et al., 2009). Interestingly, gross production rates were more strongly related to total soil C mineralisation than the fraction mineralised by methanogenesis indicating that competitive constraints on the supply of C to methanogenic communities observed in wet tropical forest soils may not apply in this environment (Teh and Silver, 2006; Dubinsky et al., 2010; Von Fischer and Hedin, 2007).

Thus, we confirm our hypothesis that methanogenic processes are active in these soils despite oxic conditions. In both the lowland forest and puna soils we find evidence to suggest that soil-atmosphere CH_4 exchange is controlled by the superficial soils. We find only partial support for the expectation that methanogenesis drives variations in the below ground CH_4 cycling. In incubations of lowland forest soils variations with depth appear to be principally associated with increases in methanotrophic activity near the surface, whilst, in the puna soils the presence of hotspots of methanogenesis may delineate source-sink activity. Due to the limited nature of these incubations it is not possible to test the causes of differences between these soil environments. However, the apparent sensitivity of methanogenesis to C availability in the puna incubations may support the assumption that variations in soil O_2 concentration increase net flux rates in this environment through the expansion of anoxic microsites and as such the quantity of C available to methanogenic processes (Burgin et al., 2011; Von Fischer and Hedin, 2007). In contrast, the lowland soils have relatively low C contents and variations in O_2 may not be sufficient to significantly influence the availability of C to methanogenic communities (Teh and Silver, 2006; Dubinsky et al., 2010). Whilst we did not conduct incubations of montane forest soils or document soil CH_4 profiles in these sites, it is interesting to note that despite the presence of thick organic horizons in the lower and upper montane forest sites we find very little evidence of CH_4 emission in measurements of soil-atmosphere CH_4 exchange. Wolf et al. (2012) have previously

documented in vitro emissions of CH_4 from such soils under oxic conditions. Contrasts between the activity of the puna and montane forests may reflect the interaction between both C availability and the potential for formation of anoxic microsites.

5.4 Broader context, limitations and future research themes

The scope for further research in constraining CH_4 cycling in the upland and montane soils of tropical South America is broad. Patterns in soil-atmosphere CH_4 exchange and below-ground CH_4 cycling discussed previously highlight key questions relating to macro, meso and micro scale variations in the interaction between these soils and the atmosphere. Similarly, variations in the drivers of soil-atmosphere CH_4 exchange and the functional components of the soil CH_4 cycle highlight fundamental questions relating to the relationship between methanogenic and methanotrophic processes. Ultimately, the suggestion that on balance the montane portion of the study region could represent a net source of CH_4 to the atmosphere highlights the need to effectively include such highlands in tropical South American CH_4 budget estimates.

At the macro or regional scale we have highlighted the source potential of humid puna grasslands and contrasting patterns of CH_4 uptake with elevation between northern and southern humid montane forests in the tropical Andes. Considering that Andean grass and shrublands extend across the entirety of the high tropical Andes, significant variability in the activity of the soils of these environments in relation to climatic variations imposed by both latitude and orography should be expected (Tovar et al., 2013). For example, we may expect the wet paramos in the northern and central tropical Andes to represent a greater source of CH_4 to the atmosphere, as these environments experience less seasonality in precipitation and as such presumably maintain optimal conditions for methanogenesis year round, than observed in our humid puna study site (Wania et al., 2009). Notably, wetland features can be significantly larger than those observed in our study region (Höfle et al., 2013). Similarly, lower precipitation and smaller soil C stocks in western and southern xeric puna systems of the tropical Andes are likely to result in smaller net CH_4 fluxes than reported for upland humid puna soils in our

study site. That said, wetland environments are still present in these xeric environments (Earle et al., 2003; Josse et al., 2009a; Otto et al., 2011). In this context, broader investigation of spatial variability in soil-atmosphere CH_4 exchange across both the uplands and wetlands of tropical Andean paramo and puna ecosystems is required. Similarly, refinements in the association between elevation and CH_4 uptake in humid montane forests of the tropical Andes are required. In particular, a focus on the relationship between orographic rainfall patterns and soil-atmosphere CH_4 exchange may prove useful in reconciling contrasting observations from these forests (Purbopuspito et al., 2006; Wolf et al., 2012).

At the meso or landscape scale we have highlighted differences in the response of soil-atmosphere CH_4 exchange for soils experiencing broadly the same climatic conditions as a result of topographic position in the humid puna, forest type in the montane forests and soil type in the lowland rainforest. However, we did not address the influence of topography on soil-atmosphere CH_4 exchange in the montane forests. These environments are typified by sharp variations in relief between valleys, slopes and ridges that potentially have implications for the soil CH_4 cycling that have not been assessed here (Silver et al., 1999; Wolf et al., 2012). In this context, broader investigations of spatial variability in soil-atmosphere CH_4 exchange as a function of topography, although a challenging prospect considering access difficulties, are required to properly constrain the activity of these forests. Similarly, the relief of these mountains results in considerable climatic variations as a function of aspect and elevation that are likely to influence soil CH_4 cycling in both the puna and montane forest landscapes.

At the micro or within landscape scale we have highlighted differences in soil CH_4 cycling within the wetlands of the humid puna and the potential for sporadic CH_4 emissions in the premontane and lowland inceptisol forests. Within the puna wetlands we considered differences between depressions containing mixed grasses and mosses and moss filled hollows, however, we did not consider the potential for emissions from pool complexes within the depressions or the numerous lakes (Fritz et al., 2011; Marani and Alvala, 2007). Similarly, we considered tussock grass dominated upland soils, however, this landscape is broken up by patches of shrubs and trees that influence soil

conditions and potential soil CH_4 cycling (Zimmermann et al., 2010b). In these contexts, broader constraints on spatial variability in soil-atmosphere CH_4 exchange from this environment should include assessments of the source-sink potential of open water features in the wetlands and variations in vegetative cover in the upland soils. Similarly, we have principally reported diffusive net CH_4 fluxes. Observation of positive soil-atmosphere CH_4 gradients in both the humid puna and some locations in the lowland inceptisol forest suggests better characterisation of transport processes is required. For example, there is the potential for larger CH_4 emissions through transport associated with aerenchymatous grasses and ebullition in the humid puna (Turetsky et al., 2014). Similarly, the potential for trees to act as conduits for CH_4 produced at depth in terra firme upland tropical forest soils has not been assessed (Pangala et al., 2013).

Contrasts between the drivers of soil-atmosphere CH_4 exchange in upland forest and puna soils has implications for generalisation of wet upland soils as sinks or sources for atmospheric CH_4. The relationship between CH_4 uptake and WFPS in the studied forests conforms to expectations used to model the activity of soil sinks, however, this is of less utility in explaining variations in CH_4 emission, driven by soil O_2 concentration, across the puna (Verchot et al., 2000; Dutaur and Verchot, 2007; Spahni et al., 2011). Similarly, across the upland soils of the transect variations in WFPS or soil O_2 concentration do not provide a convincing explanation for variations between sink and source activity. Whilst the incubation experiments indicated that the availability of C may represent a key constraint on the methanogenic activity we did not experimentally test these relationship. Future work to constrain the activity of these soils should focus on multi-factorial manipulations of water content, O_2 concentration, C availability and temperature to unpick confounding influences on the sensitivity of methanogenesis and methanotrophy (Teh et al., 2006; Teh and Silver, 2006; Dubinsky et al., 2010; Von Fischer and Hedin, 2007; Hall et al., 2013). Such information will be vital in adapting current models to realistically incorporate CH_4 emissions from non-inundated upland soils (Spahni et al., 2011). The deployment of in field pool dilution measurements within static chambers may represent a key approach to investigating the spatial drivers of hotspots of CH_4 emissions (Teh et al., 2008b; Von Fischer et al., 2009). However due the assumptions of this approach it is not likely to be valid in

montane forest soils with thick organic horizons (Nottingham et al., 2012).

Ultimately successful inclusion of Andean ecosystems into the tropical South America CH_4 budget will require appropriate estimates of landcover with which to constrain top-down and bottom-up scaling exercises. This represents a challenge, considering the apparent importance of small scale wetland features in the puna, as the resolution of current landcover maps is large (Eva et al., 2004; Josse et al., 2009b; Tovar et al., 2013). However, local assessments of variations within Andean paramo and puna environments have been made and extension of such studies will prove fruitful in moving forward from site to landscape and regional scale observations of soil-atmosphere CH_4 exchange (Otto et al., 2011; Höfle et al., 2013).

Chapter 6

Conclusions

This thesis addressed the magnitude and variability of soil-atmosphere CH_4 exchange from terra firme lowland rainforest (Chapter 2), premontane, lower montane and upper montane forest (Chapter 3) and montane humid puna grassland (Chapter 4) soils across a transition from western Amazon to high Andes in southeastern Peru. Firstly, these chapters aimed to address the paucity of information relating to the magnitude of soil sources and sinks of atmospheric CH_4 in these environments. In particular we focused on the possibility that montane environments may represent a previously unaccounted for source of atmospheric CH_4 that may help to explain discrepancies in atmospheric CH_4 budgets for tropical South America. Secondly, we aimed to investigate the drivers of variations in soil-atmosphere CH_4 exchange. In particular we focused on whether soil O_2 concentration would provide a proximal explanation for variability in these soils. Finally, in the lowland rainforest and montane grassland we aimed to investigate relationships between gross methanogenic and methanotrophic processes. In particularly we focused on whether methanogenic activity played a role in CH_4 cycling within these soils despite oxic conditions.

6.1 Key results

6.1.1 Chapter 2: Drivers of methane flux from two terra firme forest soils in the western Peruvian Amazon

This chapter aimed to investigate differences in the drivers of seasonal variability in soil-atmosphere CH_4 exchange between Amazonian terra firme tropical forests on higher porosity ultisol and lower porosity inceptisol soils and to investigate how these variations relate to the distribution of methanotrophic and methanogenic processes within these soils. Key results are as follows:

1. Both the higher porosity ultisol and lower porosity inceptisol soils studied principally acted as a sink for atmospheric CH_4 with mean net CH_4 fluxes and standard errors for the dry and wet season campaigns, respectively, of -1.59 (0.06) and -1.39 (0.07) mg CH_4-C m^{-2} d^{-1} from the ultisols and -0.95 (0.06) and -0.41 (0.10) mg CH_4-C m^{-2} d^{-1} from the inceptisols. Between season variation in net CH_4 flux from the inceptisols resulted from decreased uptake rates and an increase in the occurrence of sporadic emissions in the wet season campaign. In contrast, there was little evidence for emissions in the ultisol.

2. Greater uptake rates in the ultisol than the inceptisol were best explained, as a function of porosity, by lower WFPS. Similarly, WFPS best explained between season variation in net CH_4 flux from the inceptisol, whilst, we were unable to explain the smaller variations observed for the ultisol. We interpret these relationships as reflecting diffusional constraints on the supply of CH_4 to the high-affinity methanotrophic communities driving the dominant uptake activity of these soils.

3. Isotope pool dilution incubations indicated that methanogenic processes were active in both the ultisol and inceptisol soils despite oxic conditions. These incubations indicate uptake activity in the field was driven by high-affinity methanotrophs occupying superficial mineral soils whilst shifts in the balance between

methanogenesis and methanotrophy at depth may have driven the observed increase in emissions in the inceptisols.

4. These data highlight the complexity of biophysical constraints on soil-atmosphere CH_4 exchange from terra firme tropical forest soils. It seems likely that thresholds relating to water stresses on high-affinity methanotrophic activity and diffusional constraints on the expansion of anaerobic microsites at depth play important roles in determining how inter and intra annual climatic variations influence the strength of soil-atmosphere CH_4 exchange from such environments.

6.1.2 Chapter 3: Uptake of atmospheric methane by premontane and montane forest soils in the southern Peruvian Andes

This chapter aimed to investigate whether humid tropical montane forests of the southern Peruvian Andes functioned as a sink or source for atmospheric CH_4 and to investigate the drivers of variation in this activity in terms of elevation and seasonality. Key results are as follows:

1. The premontane, lower montane and upper montane forests principally acted as sinks for atmospheric CH_4. Mean net CH_4 fluxes and standard errors for aggregated dry and wet season months were -0.20 (0.15) and -0.08 (0.13) mg CH_4-C m^{-2} d^{-1} for the premontane forest, -1.12 (0.13) and -0.97 (0.11) mg CH_4-C m^{-2} d^{-1} for the lower montane forest and -1.55 (0.13) and -1.04 (0.11) mg CH_4-C m^{-2} d^{-1} for the upper montane forest. Soil-atmosphere exchange was predominately driven by variations in the strength of uptake in the lower and upper montane forests, whilst, emission activity contributed to net CH_4 flux in the premontane forest.

2. Increased uptake rates of atmospheric CH_4 with elevation were best explained by decreases in WFPS as a function of high porosity soils and lower mean annual precipitation. Significant variations in net CH_4 flux between seasons was only identified for the upper montane forest again reflecting the influence of seasonality on WFPS. We interpret these relationships as reflecting diffusional

constraints on the supply of CH_4 to high-affinity methanotrophic communities driving uptake of atmospheric CH_4.

3. The positive trend between uptake rate and elevation contrasts the pattern observed in the only other published study of soil-atmosphere CH_4 exchange in Andean humid tropical forests. In this context it seems likely an understanding of interactions between precipitation, soil texture and the distribution of CH_4 cycling communities in the thick organic horizons common to these forests will be required to generalise their activity.

6.1.3 Chapter 4: Methane cycling across a humid puna ecosystem in the southern Peruvian Andes

This chapter aimed to confirm the source potential of humid tropical montane grasslands through a study of an Andean humid puna ecosystem in southeastern Peru. The chapter aimed to classify soil-atmosphere CH_4 exchange and edaphic conditions for common meso and microscale landscape features and investigate the drivers of net CH_4 flux and the relationship between methanogenic and methanotrophic processes across these features. Key results are as follows:

1. The humid puna landscape exhibited considerable source potential with emissions peaking during the wet season. Mean net CH_4 fluxes and standard errors for aggregated dry and wet season months were -0.33 (0.30) and 1.30 (0.58) mg CH_4-C m^{-2} d^{-1} from the ridge, -0.64 (0.16) and 2.88 (0.60) mg CH_4-C m^{-2} d^{-1} from the slope, -0.30 (0.18) and 0.11 (0.27) mg CH_4-C m^{-2} d^{-1} from the depression and 24.65 (10.70) and 181.74 (36.35) mg CH_4-C m^{-2} d^{-1} from the hollow.

2. Characterisation of edaphic conditions indicated that uptake and emission activity was associated with both upland soils on the ridges and slopes and wetland soils in the depressions and hollows.

3. Variations in net CH_4 flux across this landscape were best explained by soil O_2 concentration reflecting the relative promotion of methanogenic activity, occurring in anoxic microsites, over methanotrophy by increases in anoxia in the upland soils.

4. Isotope pool dilution incubations indicated that methanogenic processes were active in both upland and wetland soils despite oxic conditions. These incubations indicated that the superficial soils in this landscape contain hotspots of activity favourable to the emission of CH_4. Deconvolution of gross methanogenic and methanotrophic processes indicated that variations in methanogenic activity across this landscape are driven by variations in total soil C rather than substrate competitions and that the capacity for methanotrophic activity to track production is high.

5. These data highlight the importance of considering landscape features that may act as hotspots for CH_4 cycling and suggest that soil O_2 concentrations represents a useful approach to understanding the activity of such soils as sources or sinks for atmospheric CH_4. Indication that gross methanogenic processes are constrained by total soil C rather that competitions for substrates suggests that understanding the sensitivity of methanogenic communities to variations in both O_2 and temperature may be key in understanding variability in soil-atmosphere CH_4 exchange in such environments.

6.2 Synthesis of findings

These data chapters represent key contributions to the understanding of soil-atmosphere CH_4 exchange in tropical South America as, to the authors knowledge, the second study to report on terra firme rainforest in the Peruvian lowlands (Palm et al., 2002), the second study to report on humid tropical Andean forests (Wolf et al., 2012) and the first study to report on tropical Andean montane grasslands (Teh et al., 2014). These results were discussed in Chapter 5 in terms of our focus on the possibility that montane soils may represent a previously unaccounted for source of atmospheric CH_4,

the utility of measurements of soil O_2 concentration in understanding variability soil-atmosphere CH_4 exchange across these predominately upland systems and the role played by methanogenic processes despite oxic conditions in these soils. With respect to these focuses, below we outline general conclusions for this study, the limitations of these conclusions, the implications of our findings for the broader understanding of CH_4 cycling in tropical South America and suggestions for future research priorities.

6.2.1 Conclusions

1. Andean humid puna landscapes, through the activity of both upland and wetland features, appear to have significant potential for CH_4 emissions. In contrast, Andean humid tropical forests, similarly to their lowland counterparts, appear to principally uptake atmospheric CH_4. On balance, montane landscapes in such humid Andean regions may act, driven by strong wet season emissions from wetland features, as regional sources for atmospheric CH_4.

2. Variations in soil O_2 concentration drive net CH_4 fluxes in upland soil environments such as humid puna grasslands through influence on the O_2 sensitivity of production. In contrast, net CH_4 fluxes are insensitive to variations in soil O_2 concentration in settings where soil-atmosphere exchange is governed by the activity of high-affinity methanotrophy. In such situations variations are constrained by the influence of soil texture and water content on the diffusion of atmospheric CH_4.

3. Methanogenic processes are ubiquitous in the studied soils despite the presence of oxic conditions. Differences in the relative balance between methanogenesis and methanotrophy leading to predominantly source activity from humid puna soils and sink activity in the lowland terra firme rainforest soils may relate to the availability of substrates within anoxic microsites.

6.2.2 Limitations

1. The rates of soil-atmosphere CH_4 exchange reported for the soils in this study are broadly similar to those observed in comparable environments globally. Such similarities lend confidence to our assessment of the role of these soils in the CH_4 cycle, however, our measurements are relatively poorly replicated in both space and time when considering the size of the region in question and potential climatic variability. In this respect, whilst the reported rates represent early advances in understanding the function of these ecosystems they are unlikely to fully constrain feasible spatial and temporal variability. This is particularly true for the puna grassland where data from regional analogues is currently unavailable.

2. The identified differences in drivers of soil-atmosphere CH_4 exchange from soils functioning as sources and sinks conforms to expectations regarding the constraints on microbial communities involved in the production and consumption of CH_4. However, we are unable to provide an convincing model to adequately explain variations in soil-atmosphere exchange across these soil environments. That is to say, whilst we illuminate the controls on soil-atmosphere CH_4 exchange within the studied environments we are not able to provide an empirical explanation for variations across the study transect as a whole.

3. Evidence that the studied soils support methanogenic activity despite the presence of oxic conditions is in keeping with the original motivation for the work. That is, observations of CH_4 emissions from upland soils imply the presence of in situ production. We suggest that differences in the direction and controls on soil-atmosphere CH_4 exchange between the studied humid puna and lowland rainforest may result from the ability of methanogenic organisms to exploit resources within available anoxic niches. However, we are unable to confirm this speculation as the sensitivity of gross process rates was not directly experimentally tested.

6.2.3 Impact

1. We provide the first evidence based on field observation that the humid tropical Andes, through the activity of previously unstudied puna ecosystems, are capable of acting as a net source of CH_4 to the atmosphere. The inclusion of such montane regions will aid in reconciling differences between top-down and bottom-up approaches.

2. We show that current modelling approaches, based on variations in WFPS, applied to lowland soils are appropriate in simulating soil-atmosphere CH_4 exchange in humid tropical montane forests. However, such models may fail to constrain emissions across humid puna grasslands. Similarly, wetland models reliant on water table development for source activity are inappropriate in the extensive upland portions of these ecosystems. This observation will inform model development and validation for such ecosystems as required.

3. We show that methanogenic processes are active in the principally oxic soils of both lowland terra firme rainforests and montane puna grasslands. As such we highlight the feasibility of upland soil sources, given appropriate environmental conditions, of atmospheric CH_4. As such future investigations of soil-atmosphere exchange in upland soils should not only be considered in terms of traditional measures of wetness but also the likely availability of substrates to methanogenic communities.

6.2.4 Future research priorities

1. Future research on CH_4 cycling in the tropical Andes should focus on constraining variability of soil-atmosphere CH_4 exchange in the context of the considerable variations in orography and climate found in this region. Considering the apparent importance of the humid puna, research focussing on the role of wet paramo and xeric puna ecosystems at the Andean extremes may be particularly fruitful.

2. There is scope to better constrain potentially important components of CH_4 cycling within the environments studied. For example, the presence of aerenchymatous plants and lakes in the puna indicate that non-diffusive transport pathways may be significant in determining the net source or sink activity. Equally the potential of forest soils to produce CH_4 indicates that the potential for trees to act as conduits should be investigated.

3. A better mechanistic understanding of the constraints on methanogenic activity within upland soils may help explain the function of such soils as sinks and sources of CH_4. As such, experimental work focussing on the response of gross processes to multi-factorial manipulations of water content, O_2 concentration, substrate availability and temperature is required to inform realistic model parameterisation.

4. Ultimately the successful inclusion of the Andes in the CH_4 budget for South America requires appropriate estimates of landcover. In particular, efforts should focus on constraining the extent of wetland features with the potential to act as hotspots of CH_4 emission.

Chapter 7

References

Achard, F., H. D. Eva, H. Stibig, P. Mayaux, J. Gallego, T. Richards, and J.-P. Malingreau, 2002: Determination of deforestation rates of the world's humid tropical forests. *Science*, **297**, 999–1002.

Achtnich, C., F. Bak, and R. Conrad, 1995: Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biology and Fertility of Soils*, **19**, 65–72.

Adamsen, A. and G. King, 1993: Methane consumption in temperate and subarctic forest soils: rates, vertical zonation, and responses to water and nitrogen. *Applied and Environmental Microbiology*, **59**, 485–490.

Amaral, J. A. and R. Knowles, 1995: Growth of methanotrophs in methane and oxygen counter gradients. *FEMS Microbiology Letters*, **126**, 215–220.

Andersen, B. L., G. Bidoglio, A. Leip, and D. Rembges, 1998: A new method to study simultaneous methane oxidation and methane production in soils. *Global Biogeochemical cycles*, **12**, 587–594.

Asner, G. P., C. Anderson, R. E. Martin, D. E. Knapp, R. Tupayachi, T. Kennedy-Bowdoin, F. Sinca, and Y. Malhi, 2013: Landscape-scale changes in forest structure and functional traits along an Andes-to-Amazon elevation gradient. *Biogeosciences Discussions*, **10**, 15415–15454, doi:10.5194/bgd-10-15415-2013.
URL `http://www.biogeosciences-discuss.net/10/15415/2013`

Baani, M. and W. Liesack, 2008: Two isozymes of particulate methane monooxygenase with different methane oxidation kinetics are found in methylocystis sp. strain sc2. *Proceedings of the National Academy of Sciences*, **105**, 10203–10208.

Bardgett, R. D., C. Freeman, and N. J. Ostle, 2008: Microbial contributions to climate change through carbon cycle feedbacks. *The ISME Journal*, **2**, 805–814.

Bartlett, K. B., P. M. Crill, J. A. Bonassi, J. E. Richey, and R. C. Harriss, 1990: Methane flux from the Amazon River floodplain: emissions during rising water. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **95**, 16773–16788.

Bartoń, K., 2014: *MuMIn: Multi-model inference*. R package version 1.10.5. URL `http://CRAN.R-project.org/package=MuMIn`

Bastviken, D., A. L. Santoro, H. Marotta, L. Q. Pinho, D. F. Calheiros, P. Crill, and A. Enrich-Prast, 2010: Methane emissions from Pantanal, South America, during the low water season: toward more comprehensive sampling. *Environmental Science & Technology*, **44**, 5450–5455.

Belger, L., B. R. Forsberg, and J. M. Melack, 2011: Carbon dioxide and methane emissions from interfluvial wetlands in the upper Negro River basin, Brazil. *Biogeochemistry*, **105**, 171–183.

Bender, M. and R. Conrad, 1992: Kinetics of methane oxidation in oxic soils exposed to ambient air or high methane mixing ratios. *FEMS Microbiology Letters*, **101**, 261–269.

— 1994: Methane oxidation activity in various soils and freshwater sediments: occurrence, characteristics, vertical profiles, and distribution on grain size fractions. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **99**, 16531–16540.

— 1995: Effect of methane concentrations and soil conditions on the induction of methane oxidation activity. *Soil Biology and Biochemistry*, **27**, 1517–1527.

Bergamaschi, P., C. Frankenberg, J. F. Meirink, M. Krol, M. G. Villani, S. Houweling, F. Dentener, E. J. Dlugokencky, J. B. Miller, L. V. Gatti, et al., 2009: Inverse modeling of global and regional methane emissions using SCIAMACHY satellite retrievals. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **114**, doi:10.1029/2009JD012287.

Bloom, A., P. Palmer, A. Fraser, and D. Reay, 2012: Seasonal variability of tropical wetland methane emissions: the role of the methanogen-available carbon pool. *Biogeosciences*, **9**, 2821–2830.

Bloom, A. A., J. Lee-Taylor, S. Madronich, D. J. Messenger, P. I. Palmer, D. S. Reay, and A. R. McLeod, 2010a: Global methane emission estimates from ultraviolet irradiation of terrestrial plant foliage. *New Phytologist*, **187**, 417–425.

Bloom, A. A., P. I. Palmer, A. Fraser, D. S. Reay, and C. Frankenberg, 2010b: Large-scale controls of methanogenesis inferred from methane and gravity spaceborne data. *Science*, **327**, 322–325.

Bousquet, P., P. Ciais, J. Miller, E. Dlugokencky, D. Hauglustaine, C. Prigent, G. Van der Werf, P. Peylin, E.-G. Brunke, C. Carouge, et al., 2006: Contribution of anthropogenic and natural sources to atmospheric methane variability. *Nature*, **443**, 439–443.

Bowling, D., J. Miller, M. Rhodes, S. Burns, R. Monson, and D. Baer, 2009: Soil, plant, and transport influences on methane in a subalpine forest under high ultraviolet irradiance. *Biogeosciences*, **6**, 1311–1324.

Burgin, A. J., W. H. Yang, S. K. Hamilton, and W. L. Silver, 2011: Beyond carbon and nitrogen: how the microbial energy economy couples elemental cycles in diverse ecosystems. *Frontiers in Ecology and the Environment*, **9**, 44–52.

Calhoun, A. and G. M. King, 1997: Regulation of root-associated methanotrophy by oxygen availability in the rhizosphere of two aquatic macrophytes. *Applied and Environmental Microbiology*, **63**, 3051–3058.

Carilla, J., H. R. Grau, L. Paolini, and M. Morales, 2013: Lake Fluctuations, Plant Productivity, and Long-Term Variability in High-Elevation Tropical Andean Ecosystems. *Arctic, Antarctic, and Alpine Research*, **45**, 179–189.

Chen, Y.-H. and R. G. Prinn, 2006: Estimation of atmospheric methane emissions between 1996 and 2001 using a three-dimensional global chemical transport model. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **111**, doi:10.1029/2005JD006058.

Chidthaisong, A. and R. Conrad, 2000: Turnover of glucose and acetate coupled to reduction of nitrate, ferric iron and sulfate and to methanogenesis in anoxic rice field soil. *FEMS Microbiology Ecology*, **31**, 73–86.

Chistoserdova, L., M. G. Kalyuzhnaya, and M. E. Lidstrom, 2009: The expanding world of methylotrophic metabolism. *Annual Review of Microbiology*, **63**, 477, doi:10.1146/annurev.micro.091208.073600.

Chistoserdova, L., J. A. Vorholt, and M. E. Lidstrom, 2005: A genomic view of methane oxidation by aerobic bacteria and anaerobic archaea. *Genome Biol*, **6**, 10–1186, doi:10.1186/gb-2005-6-2-208.

Cicerone, R. J. and R. S. Oremland, 1988: Biogeochemical aspects of atmospheric methane. *Global Biogeochemical Cycles*, **2**, 299–327.

Cleveland, C. C., W. R. Wieder, S. C. Reed, and A. R. Townsend, 2010: Experimental drought in a tropical rain forest increases soil carbon dioxide losses to the atmosphere. *Ecology*, **91**, 2313–2323.

Clymo, R., 1984: The limits to peat bog growth. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, **303**, 605–654.

Conrad, R., 1996: Soil microorganisms as controllers of atmospheric trace gases (hydrogen, carbon monoxide, methane, carbonyl sulfide, nitrous oxide, and nitric oxide). *Microbiological Reviews*, **60**, 609–640.

— 1999: Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, **28**, 193–202.

— 2009: The global methane cycle: recent advances in understanding the microbial processes involved. *Environmental Microbiology Reports*, **1**, 285–292.

Covey, K. R., S. A. Wood, R. J. Warren, X. Lee, and M. A. Bradford, 2012: Elevated methane concentrations in trees of an upland forest. *Geophysical Research Letters*, **39**, doi:10.1029/2012GL052361.

Curry, C. L., 2007: Modeling the soil consumption of atmospheric methane at the global scale. *Global Biogeochemical Cycles*, **21**, doi:10.1029/2006GB002818.

Dannenberg, S. and R. Conrad, 1999: Effect of rice plants on methane production and rhizospheric metabolism in paddy soil. *Biogeochemistry*, **45**, 53–71.

Davidson, E. A., F. Y. Ishida, and D. C. Nepstad, 2004: Effects of an experimental drought on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, **10**, 718–730.

Davidson, E. A., D. C. Nepstad, F. Y. Ishida, and P. M. Brando, 2008: Effects of an experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forest. *Global Change Biology*, **14**, 2582–2590.

Dedysh, S. N., C. Knief, and P. F. Dunfield, 2005: Methylocella species are facultatively methanotrophic. *Journal of Bacteriology*, **187**, 4665–4670.

Dedysh, S. N., W. Liesack, V. N. Khmelenina, N. E. Suzina, Y. A. Trotsenko, J. D. Semrau, A. M. Bares, N. S. Panikov, and J. M. Tiedje, 2000: Methylocella palustris gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs. *International Journal of Systematic and Evolutionary Microbiology*, **50**, 955–969.

Del Grosso, S., W. Parton, A. Mosier, D. Ojima, C. Potter, W. Borken, R. Brumme, K. Butterbach-Bahl, P. Crill, K. Dobbie, et al., 2000: General methane oxidation model and comparisons of methane oxidation in natural and managed systems. *Global Biogeochemical Cycles*, **14**, 999–1019.

Delmas, R., J. Servant, J. Tathy, B. Cros, and M. Labat, 1992: Sources and sinks of methane and carbon dioxide exchanges in mountain forest in equatorial Africa. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **97**, 6169–6179.

Denman, K., G. Brasseur, A. Chidthaisong, P. Ciais, P. Cox, R. Dickinson, D. Hauglustaine, C. Heinze, E. Holland, D. Jacob, U. Lohmann, S. Ramachandran, P. da Silva Dias, S. Wofsy, and X. Zhang, 2007: Couplings Between Changes in the Climate System and Biogeochemistry. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)). *Climate Change*, **2007**, 541–584.

Devol, A. H., J. E. Richey, W. A. Clark, S. L. King, and L. A. Martinelli, 1988: Methane emissions to the troposphere from the Amazon floodplain. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **93**, 1583–1592.

Devol, A. H., J. E. Richey, B. R. Forsberg, and L. A. Martinelli, 1990: Seasonal dynamics in methane emissions from the Amazon River floodplain to the troposphere. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **95**, 16417–16426.

Dlugokencky, E., L. Bruhwiler, J. White, L. Emmons, P. C. Novelli, S. A. Montzka, K. A. Masarie, P. M. Lang, A. Crotwell, J. B. Miller, et al., 2009: Observational constraints on recent increases in the atmospheric methane burden. *Geophysical Research Letters*, **36**, doi:10.1029/2009GL039780.

Dlugokencky, E., S. Houweling, L. Bruhwiler, K. Masarie, P. Lang, J. Miller, and P. Tans, 2003: Atmospheric methane levels off: Temporary pause or a new steady-state? *Geophysical Research Letters*, **30**, doi:10.1029/2003GL018126.

do Carmo, J. B., M. Keller, J. D. Dias, P. B. de Camargo, and P. Crill, 2006: A source of methane from upland forests in the brazilian amazon. *Geophysical Research Letters*, **33**, doi:10.1029/2005GL025436.

Drake, H. L., M. A. Horn, and P. K. Wüst, 2009: Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. *Environmental Microbiology Reports*, **1**, 307–318.

Dubinsky, E. A., W. L. Silver, and M. K. Firestone, 2010: Tropical forest soil microbial communities couple iron and carbon biogeochemistry. *Ecology*, **91**, 2604–2612.

Dutaur, L. and L. V. Verchot, 2007: A global inventory of the soil methane sink. *Global Biogeochemical Cycles*, **21**, doi:10.1029/2006GB002734.

Earle, L. R., B. G. Warner, and R. Aravena, 2003: Rapid development of an unusual peat-accumulating ecosystem in the Chilean Altiplano. *Quaternary Research*, **59**, 2–11.

Engle, D. and J. M. Melack, 2000: Methane emissions from an Amazon floodplain lake: Enhanced release during episodic mixing and during falling water. *Biogeochemistry*, **51**, 71–90.

Eva, H. D., A. S. Belward, E. E. De Miranda, C. M. Di Bella, V. Gond, O. Huber, S. Jones, M. Sgrenzaroli, and S. Fritz, 2004: A land cover map of South America. *Global Change Biology*, **10**, 731–744.

Feeley, K. J. and M. R. Silman, 2010: Land-use and climate change effects on population size and extinction risk of Andean plants. *Global Change Biology*, **16**, 3215–3222.

Fernandes, S. A. P., M. Bernoux, C. C. Cerri, B. J. Feigl, and M. C. Piccolo, 2002: Seasonal variation of soil chemical properties and carbon dioxide and methane fluxes in unfertilized and P-fertilized pastures in an Ultisol of the Brazilian Amazon. *Geoderma*, **107**, 227–241.

Ferry, J. G., 1993: *Fermentation of acetate*. Springer, 304–334 pp.

— 1999: Enzymology of one-carbon metabolism in methanogenic pathways. *FEMS microbiology reviews*, **23**, 13–38.

Fischer, H., M. Behrens, M. Bock, U. Richter, J. Schmitt, L. Loulergue, J. Chappellaz, R. Spahni, T. Blunier, M. Leuenberger, et al., 2008: Changing boreal methane sources and constant biomass burning during the last termination. *Nature*, **452**, 864–867.

Fisher, J. B., Y. Malhi, I. C. Torres, D. B. Metcalfe, M. J. van de Weg, P. Meir, J. E. Silva-Espejo, and W. H. Huasco, 2013: Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia*, **172**, 889–902.

Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D. Fahey, J. Haywood, J. Lean, D. Lowe, G. Myhre, J. Nganga, R. Prinn, G. Raga, M. Schulz, and R. V. Dorland, 2007: Changes in Atmospheric Constituents and in Radiative Forcing. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor and H.L. Miller (eds.)). *Climate Change*, **2007**.

Foster, P., 2001: The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Science Reviews*, **55**, 73–106.

Frankenberg, C., I. Aben, P. Bergamaschi, E. Dlugokencky, R. Van Hees, S. Houweling, P. Van Der Meer, R. Snel, and P. Tol, 2011: Global column-averaged methane mixing ratios from 2003 to 2009 as derived from SCIAMACHY: Trends and variability. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **116**, doi:10.1029/2010JD014849.

Frankenberg, C., P. Bergamaschi, A. Butz, S. Houweling, J. F. Meirink, J. Notholt, A. K. Petersen, H. Schrijver, T. Warneke, and I. Aben, 2008: Tropical methane emissions: A revised view from SCIAMACHY onboard ENVISAT. *Geophysical Research Letters*, **35**, doi:10.1029/2008GL034300.

Frankenberg, C., J. Meirink, M. Van Weele, U. Platt, and T. Wagner, 2005: Assessing methane emissions from global space-borne observations. *Science*, **308**, 1010–1014.

Frenzel, P. and E. Karofeld, 2000: Methane emission from a hollow-ridge complex in a raised bog: the role of methane production and oxidation. *Biogeochemistry*, **51**, 91–112.

Fritz, C., V. A. Pancotto, J. Elzenga, E. J. Visser, A. P. Grootjans, A. Pol, R. Iturraspe, J. G. Roelofs, and A. J. Smolders, 2011: Zero methane emission bogs: extreme rhizosphere oxygenation by cushion plants in Patagonia. *New Phytologist*, **190**, 398–408.

Gauci, V., D. J. Gowing, E. R. Hornibrook, J. M. Davis, and N. B. Dise, 2010: Woody stem methane emission in mature wetland alder trees. *Atmospheric Environment*, **44**, 2157–2160.

Gibbon, A., M. R. Silman, Y. Malhi, J. B. Fisher, P. Meir, M. Zimmermann, G. C. Dargie, W. R. Farfan, and K. C. Garcia, 2010: Ecosystem carbon storage across the grassland–forest transition in the high Andes of Manu National Park, Peru. *Ecosystems*, **13**, 1097–1111.

Gilbert, R. O., 1987: *Statistical methods for environmental pollution monitoring*. Wiley.

Girardin, C. A. J., Y. Malhi, L. Aragao, M. Mamani, W. Huaraca Huasco, L. Durand, K. Feeley, J. Rapp, J. SILVA-ESPEJO, M. Silman, et al., 2010: Net primary productivity allocation and cycling of carbon along a tropical forest elevational transect in the Peruvian Andes. *Global Change Biology*, **16**, 3176–3192.

Grant, R., 1998: Simulation of methanogenesis in the mathematical model ecosys. *Soil Biology and Biochemistry*, **30**, 883–896.

Gulledge, J. and J. P. Schimel, 1998: Low-concentration kinetics of atmospheric methane oxidation in soil and mechanism of ammonium inhibition. *Applied and Environmental Microbiology*, **64**, 4291–4298.

Hall, S. J., W. H. McDowell, and W. L. Silver, 2013: When wet gets wetter: decoupling of moisture, redox biogeochemistry, and greenhouse gas fluxes in a humid tropical forest soil. *Ecosystems*, **16**, 576–589.

Hansen, J., M. Sato, R. Ruedy, A. Lacis, and V. Oinas, 2000: Global warming in the twenty-first century: An alternative scenario. *Proceedings of the National Academy of Sciences*, **97**, 9875–9880.

Hanson, R. S. and T. E. Hanson, 1996: Methanotrophic bacteria. *Microbiological Reviews*, **60**, 439–471.

Höfle, B., L. Griesbaum, and M. Forbriger, 2013: GIS-Based Detection of Gullies in Terrestrial LiDAR Data of the Cerro Llamoca Peatland (Peru). *Remote Sensing*, **5**, 5851–5870.

Hofstede, R. G., 1995: The effects of grazing and burning on soil and plant nutrient concentrations in Colombian páramo grasslands. *Plant and Soil*, **173**, 111–132.

Holter, P., 1990: Sampling air from dung pats by silicone rubber diffusion chambers. *Soil Biology and Biochemistry*, **22**, 995–997.

Holzapfel-Pschorn, A. and W. Seiler, 1986: Methane emission during a cultivation period from an Italian rice paddy. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **91**, 11803–11814.

Hornibrook, E. and R. Aravena, 2009: *Isotopes and methane cycling, In Environmental Isotopes in Bioremediation and Biodegradation (ed. C.M. Aelion, R. Aravena, P. Hohener, and D. Hunkeler)*. CRC Press, 167–201 pp.

Hornibrook, E., H. Bowes, A. Culbert, and A. Gallego-Sala, 2009: Methanotrophy potential versus methane supply by pore water diffusion in peatlands. *Biogeosciences*, **6**, 1491–1504.

Hothorn, T., F. Bretz, and P. Westfall, 2008: Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.

Hütsch, B., 1998: Tillage and land use effects on methane oxidation rates and their vertical profiles in soil. *Biology and Fertility of Soils*, **27**, 284–292.

Ishizuka, S., A. Iswandi, Y. Nakajima, S. Yonemura, S. Sudo, H. Tsuruta, and D. Murdiyarso, 2005a: The variation of greenhouse gas emissions from soils of various land-use/cover types in Jambi province, Indonesia. *Nutrient Cycling in Agroecosystems*, **71**, 17–32.

Ishizuka, S., A. Iswandi, Y. Nakajima, S. Yonemura, S. Sudo, H. Tsuruta, and D. Muriyarso, 2005b: Spatial patterns of greenhouse gas emission in a tropical rainforest in Indonesia. *Nutrient Cycling in Agroecosystems*, **71**, 55–62.

Jacinthe, P. A. and W. A. Dick, 1996: Use of silicone tubing to sample nitrous oxide in the soil atmosphere. *Soil Biology and Biochemistry*, **28**, 721–726.

Johnson, P. S., C. L. Johnson, and N. E. West, 1988: Estimation of phytomass for ungrazed crested wheatgrass plants using allometric equations. *Journal of Range Management*, 421–425.

Josse, C., F. Cuesta, G. Navarro, V. Barrena, M. T. Becerra, E. Cabrera, E. Chacón-Moreno, W. Ferreira, M. Peralvo, J. Saito, et al., 2011: Physical geography and ecosystems in the tropical andes. *Climate Change and Biodiversity in the Tropical Andes, Inter-American Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE)*, 152–169.

Josse, C., F. Cuesta, G. Navarro, V. Barrena, E. Cabrera, E. Chacon-Moreno, W. Ferreira, M. Peralvo, J. Saito, and A. Tovar, 2009a: Ecosistemas de los Andes del norte y centro Bolivia, Colombia, Ecuador, Peru y Venezuela. *Lima: Secretaría General de la Comunidad Andina, Programa Regional ECOBONA-Intercooperation, CONDESAN-Proyecto Páramo Andino, Programa BioAndes, EcoCiencia, NatureServe, IAvH, LTAUNALM, ICAE-ULA, CDC-UNALM, and RUMBOL SRL.*.

— 2009b: Mapa de ecosistemas de los Andes del norte y centro, Bolivia, Colombia, Ecuador, Peru y Venezuela. *Lima: Secretaría General de la Comunidad Andina, Programa Regional ECOBONA-Intercooperation, CONDESAN-Proyecto Páramo Andino, Programa BioAndes, EcoCiencia, NatureServe, IAvH, LTAUNALM, ICAE-ULA, CDC-UNALM, and RUMBOL SRL.*.

Junk, W. J., 1993: Wetlands of tropical south america. *Wetlands of the world: Inventory, ecology and management Volume I*, Springer, 679–739.

Kammann, C., L. Grünhage, and H. J. Jäger, 2001: A new sampling technique to monitor concentrations of methane, nitrous oxide and carbon dioxide in air at well-defined depths in soils with varied water potential. *European Journal of Soil Science*, **52**, 297–303.

Keller, M., W. A. Kaplan, and S. C. Wofsy, 1986: Emissions of nitrous oxide, methane and carbon dioxide from tropical forest soils. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **91**, 11791–11802.

Keller, M., R. Varner, J. D. Dias, H. Silva, P. Crill, G. P. Asner, et al., 2005: Soil–Atmosphere Exchange of Nitrous Oxide, Nitric Oxide, Methane, and Carbon Dioxide in Logged and Undisturbed Forest in the Tapajos National Forest, Brazil. *Earth Interactions*, **9**, 1–21.

Keppler, F., J. T. Hamilton, M. Braß, and T. Röckmann, 2006: Methane emissions from terrestrial plants under aerobic conditions. *Nature*, **439**, 187–191.

Khalil, M. A. K., C. L. Butenhoff, and R. A. Rasmussen, 2007: Atmospheric methane: trends and cycles of sources and sinks. *Environmental Science & Technology*, **41**, 2131–2137.

Kiese, R., S. Wochele, and K. Butterbach-Bahl, 2008: Site specific and regional estimates of methane uptake by tropical rainforest soils in north eastern Australia. *Plant and soil*, **309**, 211–226.

King, G. M., 1994: Associations of methanotrophs with the roots and rhizomes of aquatic vegetation. *Applied and Environmental Microbiology*, **60**, 3220–3227.

Kip, N., J. F. van Winden, Y. Pan, L. Bodrossy, G.-J. Reichart, A. J. Smolders, M. S. Jetten, J. S. S. Damsté, and H. J. O. den Camp, 2010: Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems. *Nature Geoscience*, **3**, 617–621.

Kirschke, S., P. Bousquet, P. Ciais, M. Saunois, J. G. Canadell, E. J. Dlugokencky, P. Bergamaschi, D. Bergmann, D. R. Blake, L. Bruhwiler, et al., 2013: Three decades of global methane sources and sinks. *Nature Geoscience*, **6**, 813–823.

Klute, A. et al., 1986: *Methods of soil analysis. Part 1. Physical and mineralogical methods.*. Number Ed. 2, American Society of Agronomy, Inc.

Knief, C., S. Kolb, P. L. Bodelier, A. Lipski, and P. F. Dunfield, 2006: The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. *Environmental Microbiology*, **8**, 321–333.

Knief, C., A. Lipski, and P. F. Dunfield, 2003: Diversity and activity of methanotrophic bacteria in different upland soils. *Applied and Environmental Microbiology*, **69**, 6703–6714.

Kolb, S., 2009: The quest for atmospheric methane oxidizers in forest soils. *Environmental Microbiology Reports*, **1**, 336–346.

Kolb, S., C. Knief, P. F. Dunfield, and R. Conrad, 2005: Abundance and activity of uncultured methanotrophic bacteria involved in the consumption of atmospheric methane in two forest soils. *Environmental Microbiology*, **7**, 1150–1161.

Kursar, T. A., S. J. Wright, and R. Radulovich, 1995: The effects of the rainy season and irrigation on soil water and oxygen in a seasonal forest in Panama. *Journal of Tropical Ecology*, **11**, 497–515.

Le Mer, J. and P. Roger, 2001: Production, oxidation, emission and consumption of methane by soils: a review. *European Journal of Soil Biology*, **37**, 25–50.

Lelieveld, J., P. J. Crutzen, and F. J. Dentener, 1998: Changing concentration, lifetime and climate forcing of atmospheric methane. *Tellus B*, **50**, 128–150.

Lima, I. B. T., 2005: Biogeochemical distinction of methane releases from two amazon hydroreservoirs. *Chemosphere*, **59**, 1697–1702.

Liptzin, D. and W. L. Silver, 2009: Effects of carbon additions on iron reduction and phosphorus availability in a humid tropical forest soil. *Soil Biology and Biochemistry*, **41**, 1696–1702.

Liptzin, D., W. L. Silver, and M. Detto, 2011: Temporal dynamics in soil oxygen and greenhouse gases in two humid tropical forests. *Ecosystems*, **14**, 171–182.

Liu, Y. and W. B. Whitman, 2008: Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, **1125**, 171–189.

Livingston, G. and G. Hutchinston, 1995: *Chapter 2: Enclosure-based measurement of trace gas exchange: applications and sources of error. In: Matson, P., Harriss, RC (ed.) Biogenic Trace Gases: Measuring Emissions from Soil and Water*. Blackwell Scientific Ltd.

Loulergue, L., A. Schilt, R. Spahni, V. Masson-Delmotte, T. Blunier, B. Lemieux, J.-M. Barnola, D. Raynaud, T. F. Stocker, and J. Chappellaz, 2008: Orbital and millennial-scale features of atmospheric methane over the past 800,000 years. *Nature*, **453**, 383–386.

Lovley, D. R., D. F. Dwyer, and M. J. Klug, 1982: Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Applied and Environmental Microbiology*, **43**, 1373–1379.

Lovley, D. R. and E. J. Phillips, 1987: Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. *Applied and Environmental Microbiology*, **53**, 2636–2641.

Luteyn, J. L. and S. P. Churchill, 1999: *Páramos: a checklist of plant diversity, geographical distribution, and botanical literature*. New York Botanical Garden Press New York.

Malhi, Y., F. Farfán Amézquita, C. E. Doughty, J. E. Silva-Espejo, C. A. Girardin, D. B. Metcalfe, L. E. Aragão, L. P. Huaraca-Quispe, I. Alzamora-Taype, L. Eguiluz-Mora, et al., 2013: The productivity, metabolism and carbon cycle of two lowland tropical forest plots in south-western Amazonia, Peru. *Plant Ecology & Diversity*, **7**, 85–105, doi:10.1080/17550874.2013.820805.

Malhi, Y., M. Silman, N. Salinas, M. Bush, P. Meir, and S. Saatchi, 2010: Introduction: elevation gradients in the tropics: laboratories for ecosystem ecology and global change research. *Global Change Biology*, **16**, 3171–3175.

Marani, L. and P. Alvala, 2007: Methane emissions from lakes and floodplains in Pantanal, Brazil. *Atmospheric Environment*, **41**, 1627–1633.

Martinson, G. O., F. A. Werner, C. Scherber, R. Conrad, M. D. Corre, H. Flessa, K. Wolf, M. Klose, S. R. Gradstein, and E. Veldkamp, 2010: Methane emissions from tank bromeliads in neotropical forests. *Nature Geoscience*, **3**, 766–769.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, et al., 2003: Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. *Ecosystems*, **6**, 301–312.

McLeod, A. R., S. C. Fry, G. J. Loake, D. J. Messenger, D. S. Reay, K. A. Smith, and B.-W. Yun, 2008: Ultraviolet radiation drives methane emissions from terrestrial plant pectins. *New Phytologist*, **180**, 124–132.

McNamara, N. P., T. Plant, S. Oakley, S. Ward, C. Wood, and N. Ostle, 2008: Gully hotspot contribution to landscape methane and carbon dioxide fluxes in a northern peatland. *Science of the Total Environment*, **404**, 354–360.

Megonigal, J. P. and A. B. Guenther, 2008: Methane emissions from upland forest soils and vegetation. *Tree Physiology*, **28**, 491–498.

Melack, J. M., L. L. Hess, M. Gastil, B. R. Forsberg, S. K. Hamilton, I. B. Lima, and E. M. Novo, 2004: Regionalization of methane emissions in the Amazon Basin with microwave remote sensing. *Global Change Biology*, **10**, 530–544.

Mikaloff Fletcher, S. E., P. P. Tans, L. M. Bruhwiler, J. B. Miller, and M. Heimann, 2004a: Methane sources estimated from atmospheric observations of methane and its 13C/12C isotopic ratios: 1. Inverse modeling of source processes. *Global Biogeochemical Cycles*, **18**, doi:10.1029/2004GB002223.

— 2004b: Methane sources estimated from atmospheric observations of methane and its 13C/12C isotopic ratios: 2. Inverse modeling of CH4 fluxes from geographical regions. *Global Biogeochemical Cycles*, **18**, doi:10.1029/2004GB002224.

Miller, D. C. and P. W. Birkeland, 1992: Soil catena variation along an alpine climatic transect, northern peruvian andes. *Geoderma*, **55**, 211–223.

Miller, J. B., L. V. Gatti, M. T. d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio d'Amelio, A. M. Crotwell, E. J. Dlugokencky, P. Bakwin, P. Artaxo, and P. P. Tans, 2007: Airborne measurements indicate large methane emissions from the eastern Amazon basin. *Geophysical Research Letters*, **34**, doi:10.1029/2006GL029213.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. Da Fonseca, and J. Kent, 2000: Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Nakagawa, S. and H. Schielzeth, 2013: A general and simple method for obtaining r2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133–142.

Neto, E. S., J. Carmo, M. Keller, S. Martins, L. Alves, S. Vieira, M. Piccolo, P. Camargo, H. Couto, C. Joly, et al., 2011: Soil-atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest. *Biogeosciences*, **8**, 733–742.

Nisbet, E. and J. Chappellaz, 2009: Shifting gear, quickly. *Science*, **324**, 477–478.

Nisbet, E. G., E. J. Dlugokencky, and P. Bousquet, 2014: Methane on the rise-again. *Science*, **343**, 493–495.

Nottingham, A. T., A. J. Ccahuana, and P. Meir, 2012: Soil properties in tropical montane cloud forests influence estimates of soil carbon dioxide efflux. *Agricultural and Forest Meteorology*, **166**, 215–220.

Noyce, G. L., R. K. Varner, J. L. Bubier, and S. Frolking, 2014: Effect of carex rostrata on seasonal and interannual variability in peatland methane emissions. *Journal of Geophysical Research: Biogeosciences*, **119**, 24–34.

O'Connor, F. M., O. Boucher, N. Gedney, C. Jones, G. Folberth, R. Coppell, P. Friedlingstein, W. Collins, J. Chappellaz, J. Ridley, et al., 2010: Possible role of wetlands, permafrost, and methane hydrates in the methane cycle under future climate change: A review. *Reviews of Geophysics*, **48**, doi:10.1029/2010RG000326.

Oliveras, I., L. O. Anderson, and Y. Malhi, 2014a: Application of remote sensing to understanding fire regimes and biomass burning emissions of the tropical Andes. *Global Biogeochemical Cycles*, **28**, 480–496.

Oliveras, I., M. Eynden, Y. Malhi, N. Cahuana, C. Menor, F. Zamora, and T. Haugaasen, 2014b: Grass allometry and estimation of above-ground biomass in tropical alpine tussock grasslands. *Austral Ecology*, **39**, 408–415.

Op den Camp, H. J., T. Islam, M. B. Stott, H. R. Harhangi, A. Hynes, S. Schouten, M. S. Jetten, N.-K. Birkeland, A. Pol, and P. F. Dunfield, 2009: Environmental, genomic and taxonomic perspectives on methanotrophic verrucomicrobia. *Environmental Microbiology Reports*, **1**, 293–306.

Otto, M., D. Scherer, and J. Richters, 2011: Hydrological differentiation and spatial distribution of high altitude wetlands in a semi-arid andean region derived from satellite data. *Hydrology and Earth System Sciences*, **15**, 1713–1727.

Page, S. E., J. O. Rieley, and C. J. Banks, 2011: Global and regional importance of the tropical peatland carbon pool. *Global Change Biology*, **17**, 798–818.

Palm, C., J. Alegre, L. Arevalo, P. Mutuo, A. Mosier, and R. Coe, 2002: Nitrous oxide and methane fluxes in six different land use systems in the Peruvian Amazon. *Global Biogeochemical Cycles*, **16**, 21–1, doi:10.1029/2001GB001855.

Pangala, S. R., S. Moore, E. R. Hornibrook, and V. Gauci, 2013: Trees are major conduits for methane egress from tropical forested wetlands. *New Phytologist*, **197**, 524–531.

Pansu, M., L. Sarmiento, K. Metselaar, D. Hervé, and P. Bottner, 2007: Modelling the transformations and sequestration of soil organic matter in two contrasting ecosystems of the Andes. *European Journal of Soil Science*, **58**, 775–785.

Parkin, T., R. Venterea, and S. Hargreaves, 2012: Calculating the detection limits of chamber-based soil greenhouse gas flux measurements. *Journal of Environmental Quality*, **41**, 705–715.

Pedersen, A. R., 2012: *HMR: Flux estimation with static chamber data*. R package version 0.3.1.
URL `http://cran.r-project.org/package=HMR`

Pedersen, A. R., S. O. Petersen, and K. Schelde, 2010: A comprehensive approach to soil-atmosphere trace-gas flux estimation with static chambers. *European Journal of Soil Science*, **61**, 888–902.

Peters, V. and R. Conrad, 1996: Sequential reduction processes and initiation of methane production upon flooding of oxic upland soils. *Soil Biology and Biochemistry*, **28**, 371–382.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team, 2014: *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-117.
URL `http://CRAN.R-project.org/package=nlme`

Pinheiro, J. C. and D. M. Bates, 2000: *Mixed-effects models in S and S-PLUS*. Springer.

Potter, C., E. Davidson, and L. Verchot, 1996: Estimation of global biogeochemical controls and seasonality in soil methane consumption. *Chemosphere*, **32**, 2219–2246.

Priemé, A. and S. Christensen, 1997: Seasonal and spatial variation of methane oxidation in a Danish spruce forest. *Soil Biology and Biochemistry*, **29**, 1165–1172.

Pumpanen, J., P. Kolari, H. Ilvesniemi, K. Minkkinen, T. Vesala, S. Niinistö, A. Lohila, T. Larmola, M. Morero, M. Pihlatie, et al., 2004: Comparison of different chamber techniques for measuring soil carbon dioxide efflux. *Agricultural and Forest Meteorology*, **123**, 159–176.

Purbopuspito, J., E. Veldkamp, R. Brumme, and D. Murdiyarso, 2006: Trace gas fluxes and nitrogen cycling along an elevation sequence of tropical montane forests in Central Sulawesi, Indonesia. *Global Biogeochemical Cycles*, **20**, doi:10.1029/2005GB002516.

Querino, C., C. Smeets, I. Vigano, R. Holzinger, V. Moura, L. Gatti, A. Martinewski, A. Manzi, A. De Araújo, and T. Röckmann, 2011: Methane flux, vertical gradient and mixing ratio measurements in a tropical forest. *Atmospheric Chemistry and Physics*, **11**, 7943–7953.

R Core Team, 2013: *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
URL http://www.r-project.org

Raghoebarsing, A. A., A. J. Smolders, M. C. Schmid, W. I. C. Rijpstra, M. Wolters-Arts, J. Derksen, M. S. Jetten, S. Schouten, J. S. S. Damsté, L. P. Lamers, et al., 2005: Methanotrophic symbionts provide carbon for photosynthesis in peat bogs. *Nature*, **436**, 1153–1156.

Reay, D. S. and D. B. Nedwell, 2004: Methane oxidation in temperate soils: effects of inorganic N. *Soil Biology and Biochemistry*, **36**, 2059–2065.

Rehm, E. M. and K. J. Feeley, 2013: Forest patches and the upward migration of timberline in the southern Peruvian Andes. *Forest Ecology and Management*, **305**, 204–211.

Richey, J. E., A. H. Devol, S. C. Wofsy, R. Victoria, and M. N. Riberio, 1988: Biogenic gases and the oxidation and reduction of carbon in Amazon River and floodplain waters. *Limnol. Oceanogr*, **33**, 551–561.

Rigby, M., R. G. Prinn, P. J. Fraser, P. G. Simmonds, R. Langenfelds, J. Huang, D. M. Cunnold, L. P. Steele, P. B. Krummel, R. F. Weiss, et al., 2008: Renewed growth of atmospheric methane. *Geophysical Research Letters*, **35**, doi:10.1029/2008GL036037.

Ringeval, B., N. de Noblet-Ducoudré, P. Ciais, P. Bousquet, C. Prigent, F. Papa, and W. B. Rossow, 2010: An attempt to quantify the impact of changes in wetland extent on methane emissions on the seasonal and interannual time scales. *Global Biogeochemical Cycles*, **24**, doi:10.1029/2008GB003354.

Sawakuchi, H. O., D. Bastviken, A. O. Sawakuchi, A. V. Krusche, M. V. R. Ballester, and J. E. Richey, 2014: Methane emissions from Amazonian Rivers and their contribution to the global methane budget. *Global Change Biology*, doi:10.1111/gcb.12646.

Schimel, J. P., 1995: Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. *Biogeochemistry*, **28**, 183–200.

Schuur, E. A., O. A. Chadwick, and P. A. Matson, 2001: Carbon cycling and soil carbon storage in mesic to wet Hawaiian montane forests. *Ecology*, **82**, 3182–3196.

Segers, R., 1998: Methane production and methane consumption: a review of processes underlying wetland methane fluxes. *Biogeochemistry*, **41**, 23–51.

Segnini, A., A. A. de Souza, E. H. Novotny, D. M. B. P. Milori, W. T. L. da Silva, T. J. Bonagamba, A. Posadas, and R. Quiroz, 2013: Characterization of Peatland Soils from the High Andes through C Nuclear Magnetic Resonance Spectroscopy. *Soil Science Society of America Journal*, **77**, 673–679.

Sexstone, A. and C. Mains, 1990: Production of methane and ethylene in organic horizons of spruce forest soils. *Soil Biology and Biochemistry*, **22**, 135–139.

Sexstone, A. J., N. P. Revsbech, T. B. Parkin, and J. M. Tiedje, 1985: Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal*, **49**, 645–651.

Shakhova, N., I. Semiletov, A. Salyuk, V. Yusupov, D. Kosmach, and Ö. Gustafsson, 2010: Extensive methane venting to the atmosphere from sediments of the East Siberian Arctic Shelf. *Science*, **327**, 1246–1250.

Shannon, R. D., J. R. White, J. E. Lawson, and B. S. Gilmour, 1996: Methane efflux from emergent vegetation in peatlands. *Journal of Ecology*, 239–246.

Shindell, D. T., G. Faluvegi, N. Bell, and G. A. Schmidt, 2005: An emissions-based view of climate forcing by methane and tropospheric ozone. *Geophysical Research Letters*, **32**, doi:10.1029/2004GL021900.

Silver, W. L., A. Lugo, and M. Keller, 1999: Soil oxygen availability and biogeochemistry along rainfall and topographic gradients in upland wet tropical forest soils. *Biogeochemistry*, **44**, 301–328.

Singarayer, J. S., P. J. Valdes, P. Friedlingstein, S. Nelson, and D. J. Beerling, 2011: Late holocene methane rise caused by orbitally controlled increase in tropical sources. *Nature*, **470**, 82–85.

Singh, B. K., R. D. Bardgett, P. Smith, and D. S. Reay, 2010: Microorganisms and climate change: terrestrial feedbacks and mitigation options. *Nature Reviews Microbiology*, **8**, 779–790.

Sinha, V., J. Williams, P. Crutzen, and J. Lelieveld, 2007: Methane emissions from boreal and tropical forest ecosystems derived from in-situ measurements. *Atmospheric Chemistry and Physics Discussions*, **7**, 14011–14039.

Sjögersten, S., C. R. Black, S. Evers, J. Hoyos-Santillan, E. L. Wright, and B. L. Turner, 2014: Tropical wetlands: A missing link in the global carbon cycle? *Global Biogeochemical Cycles*, doi:10.1002/2014GB004844.

Sjögersten, S., E. Melander, and P. A. Wookey, 2007: Depth Distribution of Net Methanotrophic Activity at a Mountain Birch Forest-Tundra Heath Ecotone, Northern Sweden. *Arctic, Antarctic, and Alpine Research*, **39**, 477–480.

Smith, K., T. Ball, F. Conen, K. Dobbie, J. Massheder, and A. Rey, 2003: Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, **54**, 779–791.

Smith, K., K. Dobbie, B. Ball, L. Bakken, B. Sitaula, S. Hansen, R. Brumme, W. Borken, S. Christensen, A. Priemé, et al., 2000a: Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Global Change Biology*, **6**, 791–803.

Smith, L. K., W. M. Lewis Jr, J. P. Chanton, G. Cronin, and S. K. Hamilton, 2000b: Methane emissions from the orinoco river floodplain, venezuela. *Biogeochemistry*, **51**, 113–140.

Spahni, R., J. Chappellaz, T. F. Stocker, L. Loulergue, G. Hausammann, K. Kawamura, J. Flückiger, J. Schwander, D. Raynaud, V. Masson-Delmotte, et al., 2005: Atmospheric methane and nitrous oxide of the late pleistocene from antarctic ice cores. *Science*, **310**, 1317–1321.

Spahni, R., R. Wania, L. Neef, M. Van Weele, I. Pison, P. Bousquet, C. Frankenberg, P. Foster, F. Joos, I. Prentice, et al., 2011: Constraining global methane emissions and uptake by ecosystems. *Biogeosciences Discussions*, **8**, doi:10.5194/bg-8-1643-2011.

Sparks, D. L., A. Page, P. Helmke, R. Loeppert, P. Soltanpour, M. Tabatabai, C. Johnston, M. Sumner, et al., 1996: *Methods of soil analysis. Part 3-Chemical methods.*. Soil Science Society of America Inc.

Squeo, F. A., B. G. Warner, R. Aravena, and D. Espinoza, 2006: Bofedales: high altitude peatlands of the central Andes. *Revista Chilena de Historia Natural*, **79**, 245–255.

Steudler, P. A., J. M. Melillo, B. J. Feigl, C. Neill, M. C. Piccolo, and C. C. Cerri, 1996: Consequence of forest-to-pasture conversion on methane fluxes in the Brazilian Amazon Basin. *Journal of Geophysical Research: Atmospheres (1984–2012)*, **101**, 18547–18554.

Ström, L., A. Ekberg, M. Mastepanov, and T. Røjle Christensen, 2003: The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biology*, **9**, 1185–1192.

Sundh, I., M. Nilsson, G. Granberg, and B. Svensson, 1994: Depth distribution of microbial production and oxidation of methane in northern boreal peatlands. *Microbial Ecology*, **27**, 253–265.

Teh, Y., T. Diem, S. Jones, L. H. Quispe, E. Baggs, N. Morley, M. Richards, P. Smith, and P. Meir, 2014: Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes. *Biogeosciences*, **11**, 2325–2339.

Teh, Y. A., E. A. Dubinsky, W. L. Silver, and C. M. Carlson, 2008a: Suppression of methanogenesis by dissimilatory iron (III)-reducing bacteria in tropical rain forest soils: Implications for ecosystem methane flux. *Global Change Biology*, **14**, 413–422.

Teh, Y. A., R. C. Rhew, A. Atwood, and T. Abel, 2008b: Water, temperature, and vegetation regulation of methyl chloride and methyl bromide fluxes from a shortgrass steppe ecosystem. *Global Change biology*, **14**, 77–91.

Teh, Y. A. and W. L. Silver, 2006: Effects of soil structure destruction on methane production and carbon partitioning between methanogenic pathways in tropical rain forest soils. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, **111**, doi:10.1029/2005JG000020.

Teh, Y. A., W. L. Silver, and M. E. Conrad, 2005: Oxygen effects on methane production and oxidation in humid tropical forest soils. *Global Change Biology*, **11**, 1283–1297.

Teh, Y. A., W. L. Silver, M. E. Conrad, S. E. Borglin, and C. M. Carlson, 2006: Carbon isotope fractionation by methane-oxidizing bacteria in tropical rain forest soils. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, **111**, doi:10.1029/2005JG000053.

Teh, Y. A., W. L. Silver, O. Sonnentag, M. Detto, M. Kelly, and D. D. Baldocchi, 2011: Large greenhouse gas emissions from a temperate peatland pasture. *Ecosystems*, **14**, 311–325.

Terazawa, K., S. Ishizuka, T. Sakata, K. Yamada, and M. Takahashi, 2007: Methane emissions from stems of fraxinus mandshurica var. japonica trees in a floodplain forest. *Soil Biology and Biochemistry*, **39**, 2689–2692.

Tovar, C., C. A. Arnillas, F. Cuesta, and W. Buytaert, 2013: Diverging responses of tropical Andean biomes under future climate conditions. *PloS One*, **8**, e63634, doi:10.1371/journal.pone.0063634.

Turetsky, M. R., A. Kotowska, J. Bubier, N. B. Dise, P. Crill, E. R. Hornibrook, K. Minkkinen, T. R. Moore, I. H. Myers-Smith, H. Nykänen, et al., 2014: A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. *Global Change Biology*, doi:10.1111/gcb.12580.

van de Weg, M. J., P. Meir, J. Grace, and G. D. Ramos, 2012: Photosynthetic parameters, dark respiration and leaf traits in the canopy of a peruvian tropical montane cloud forest. *Oecologia*, **168**, 23–34.

Varadhan, R. and P. Gilbert, 2014: *BB: Solving and Optimizing Large-Scale Nonlinear Systems*. R package version 2014.1-1.
URL `http://cran.r-project.org/package=BB`

Varner, R. K., M. Keller, J. R. Robertson, J. D. Dias, H. Silva, P. M. Crill, M. McGroddy, and W. L. Silver, 2003: Experimentally induced root mortality increased nitrous oxide emission from tropical forest soils. *Geophysical Research Letters*, **30**, 1144, doi:10.1029/2002GL016164.

Verchot, L. V., E. A. Davidson, J. H. Cattânio, and I. L. Ackerman, 2000: Land-use change and biogeochemical controls of methane fluxes in soils of eastern Amazonia. *Ecosystems*, **3**, 41–56.

Von Fischer, J. C., G. Butters, P. C. Duchateau, R. J. Thelwell, and R. Siller, 2009: In situ measures of methanotroph activity in upland soils: A reaction-diffusion model and field observation of water stress. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, **114**, doi:10.1029/2008JG000731.

Von Fischer, J. C. and L. O. Hedin, 2002: Separating methane production and consumption with a field-based isotope pool dilution technique. *Global Biogeochemical Cycles*, **16**, 1034, doi:10.1029/2001GB001448.

— 2007: Controls on soil methane fluxes: Tests of biophysical mechanisms using stable isotope tracers. *Global Biogeochemical Cycles*, **21**, doi:10.1029/2006GB002687.

Von Fischer, J. C., R. C. Rhew, G. M. Ames, B. K. Fosdick, and P. E. von Fischer, 2010: Vegetation height and other controls of spatial variability in methane emissions from the Arctic coastal tundra at Barrow, Alaska. *Journal of Geophysical Research: Biogeosciences (2005–2012)*, **115**, doi:10.1029/2009JG001283.

Wachinger, G., S. Fiedler, K. Zepp, A. Gattinger, M. Sommer, and K. Roth, 2000: Variability of soil methane production on the micro-scale: spatial association with hot spots of organic material and archaeal populations. *Soil Biology and Biochemistry*, **32**, 1121–1130.

Waddington, J. and N. Roulet, 1996: Atmosphere-wetland carbon exchanges: Scale dependency of carbon dioxide and methane exchange on the developmental topography of a peatland. *Global Biogeochemical Cycles*, **10**, 233–245.

Walter, B. P. and M. Heimann, 2000: A process-based, climate-sensitive model to derive methane emissions from natural wetlands: Application to five wetland sites, sensitivity to model parameters, and climate. *Global Biogeochemical Cycles*, **14**, 745–765.

Walter, K. M., S. Zimov, J. P. Chanton, D. Verbyla, and F. S. Chapin, 2006: Methane bubbling from siberian thaw lakes as a positive feedback to climate warming. *Nature*, **443**, 71–75.

Wania, R., M. Jolleys, and W. Buytaert, 2009: A previously neglected methane source from the andean paramo? *iLEAPS Newsletter*, **7**, 58–59.

Werner, C., X. Zheng, J. Tang, B. Xie, C. Liu, R. Kiese, and K. Butterbach-Bahl, 2006: Nitrous oxide, methane and carbon dioxide emissions from seasonal tropical rainforests and a rubber plantation in Southwest China. *Plant and Soil*, **289**, 335–353.

Whitaker, J., N. Ostle, A. T. Nottingham, A. Ccahuana, N. Salinas, R. D. Bardgett, P. Meir, and N. P. McNamara, 2014: Microbial community composition explains soil respiration responses to changing carbon inputs along an Andes-to-Amazon elevation gradient. *Journal of Ecology*, doi:10.1111/1365-2745.12247.

Whiting, G. and J. Chanton, 1993: Primary production control of methane emission from wetlands. *Nature 364*, **364**, 794–795.

Wolf, K., H. Flessa, and E. Veldkamp, 2012: Atmospheric methane uptake by tropical montane forest soils and the contribution of organic layers. *Biogeochemistry*, **111**, 469–483.

Wolff, E. W., 2011: Global change: methane and monsoons. *Nature*, **470**, 49–50.

Wu, L., 2009: *Mixed effects models for complex data*. CRC Press.

Yavitt, J., D. Downey, G. Lang, and A. Sexston, 1990: Methane consumption in two temperate forest soils. *Biogeochemistry*, **9**, 39–52.

Zimmermann, M., P. Meir, M. Bird, Y. Malhi, and A. Ccahuana, 2009: Climate dependence of heterotrophic soil respiration from a soil-translocation experiment along a 3000 m tropical forest altitudinal gradient. *European Journal of Soil Science*, **60**, 895–906.

Zimmermann, M., P. Meir, M. I. Bird, Y. Malhi, and A. J. Ccahuana, 2010a: Temporal variation and climate dependence of soil respiration and its components along a 3000 m altitudinal tropical forest gradient. *Global Biogeochemical Cycles*, **24**, doi:10.1029/2010GB003787.

Zimmermann, M., P. Meir, M. R. Silman, A. Fedders, A. Gibbon, Y. Malhi, D. H. Urrego, M. B. Bush, K. J. Feeley, K. C. Garcia, et al., 2010b: No differences in soil carbon stocks across the tree line in the Peruvian Andes. *Ecosystems*, **13**, 62–74.

Zinder, S. H., 1993: Physiological ecology of methanogens. *Methanogenesis*, Springer, 128–206.

Zuur, A., E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith, 2009: *Mixed effects models and extensions in ecology with R*. Springer.

Zuur, A. F., E. N. Ieno, and G. M. Smith, 2007: *Analysing ecological data*, volume 680. Springer New York.

Appendix A

Data examples for the isotope pool dilution model

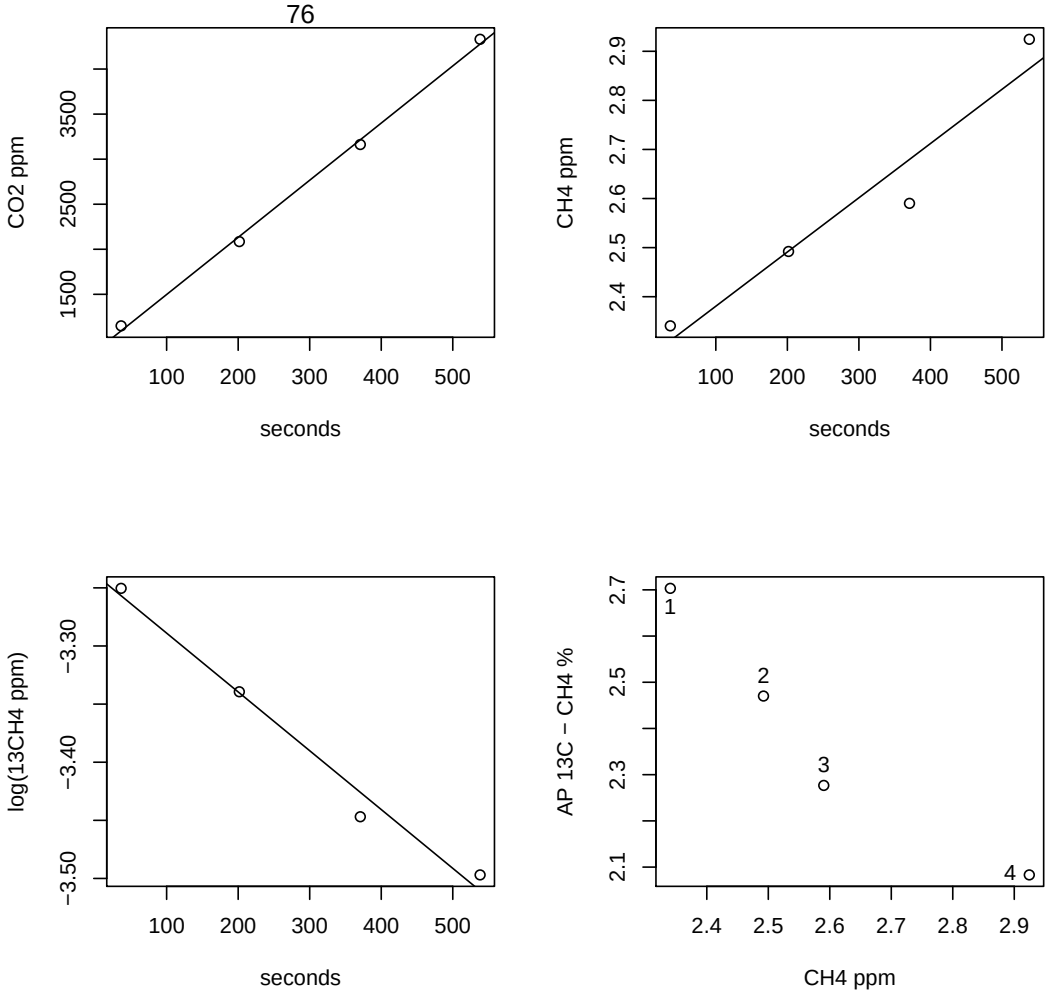

Figure A.1: Data fits for an incubation where P > C. The amount of CH_4 increases with time and a negative relationship is observed between amount of CH_4 and atom percent of $^{13}CH_4$ as the headspace is diluted by isotopically light CH_4

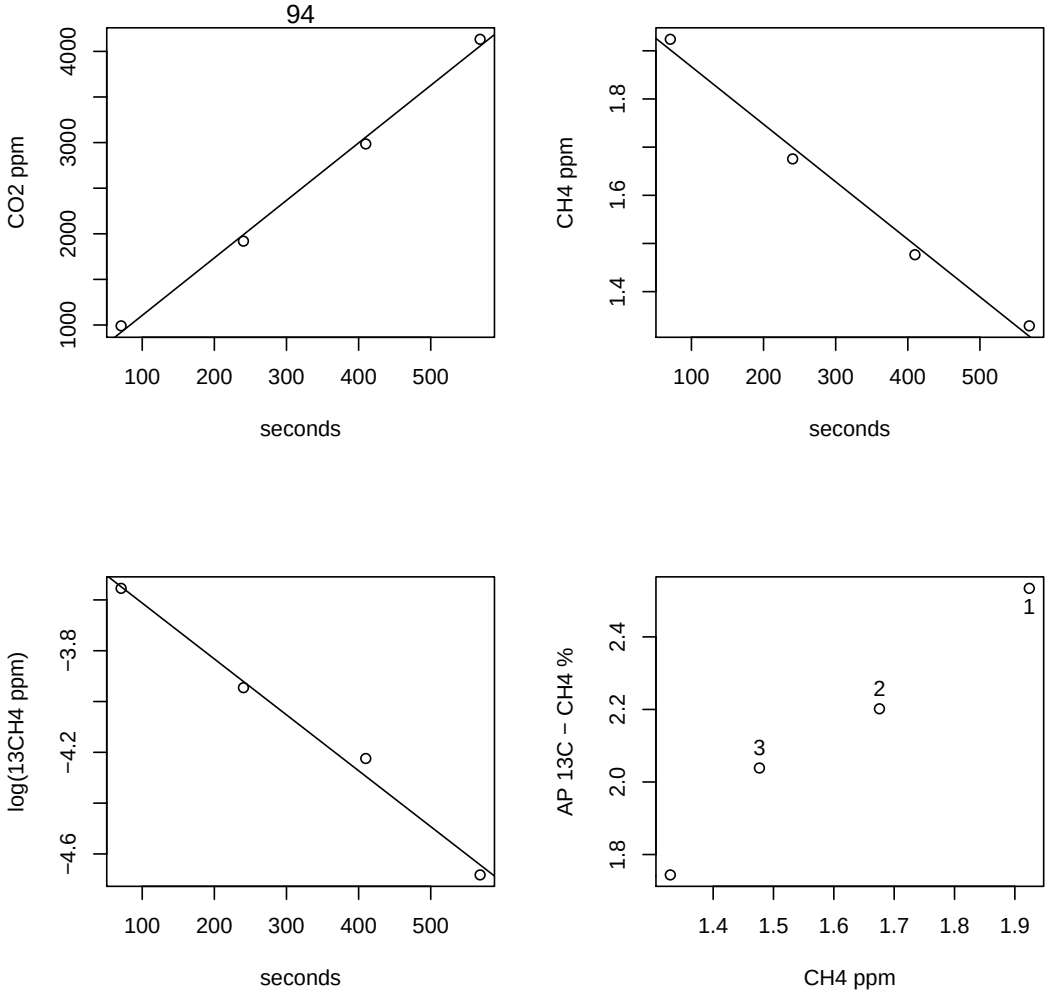

Figure A.2: Data fits for an incubation where C > P. The amount of CH_4 decreases with time and a positive relationship is observed between amount of CH_4 and atom percent of $^{13}CH_4$ as $^{12}CH_4$ is preferentially consumed with respect to $^{13}CH_4$.

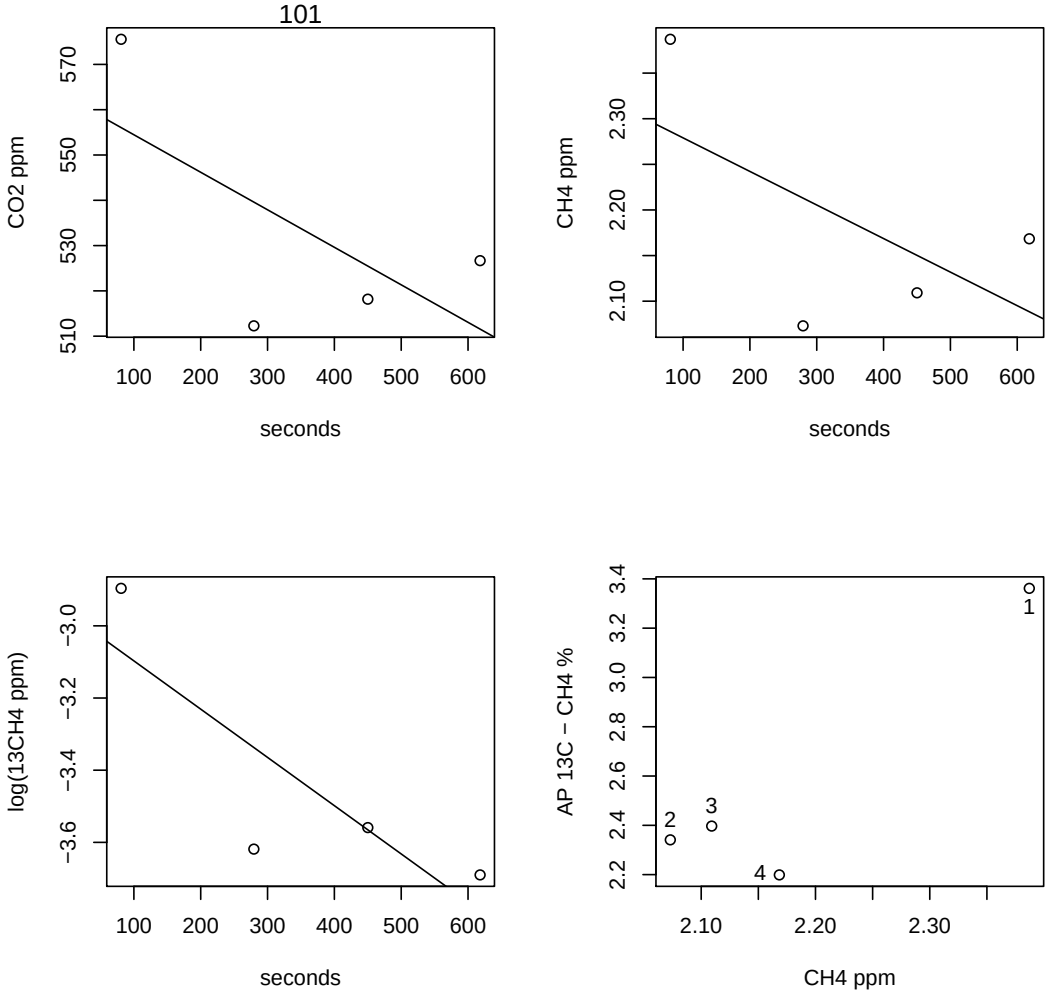

Figure A.3: Data fits for a blank incubation. No significant trends with time observed for amount of CH_4 or atom percent of $^{13}CH_4$. Variations associated with analysis and sampling are used to inform error minimisation in iteratively solving for P and C.

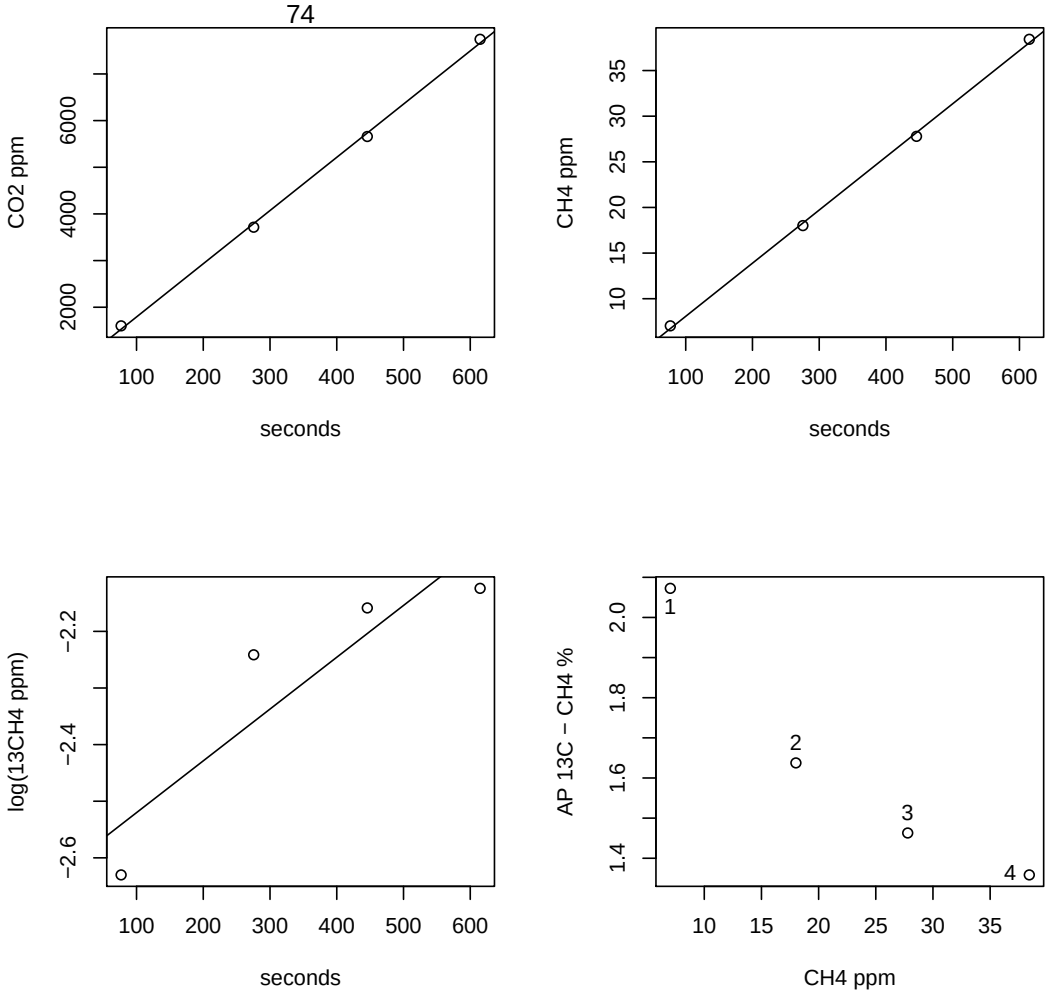

Figure A.4: Data fits for an incubation where high cycling rates invalidated the assumptions of the model. In this situation the amount of labelled CH_4 in the headspace increases with time indicating that the contribution of $^{13}CH_4$ from high levels of production is not insignificant.

Appendix B

Supplementary data for Chapter 2

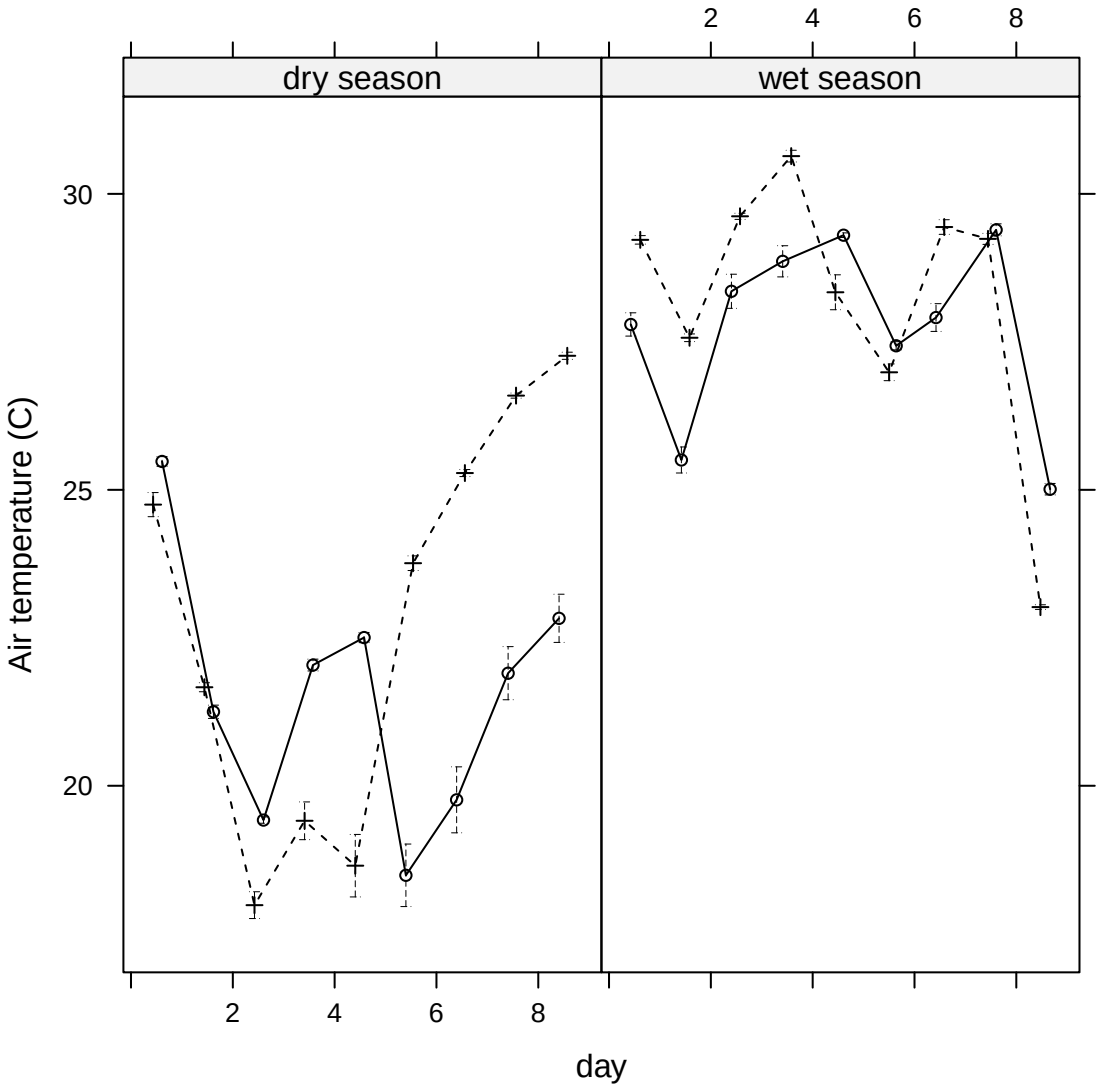

Figure B.1: Daily averaged air temperature for both sites during dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars standard errors.

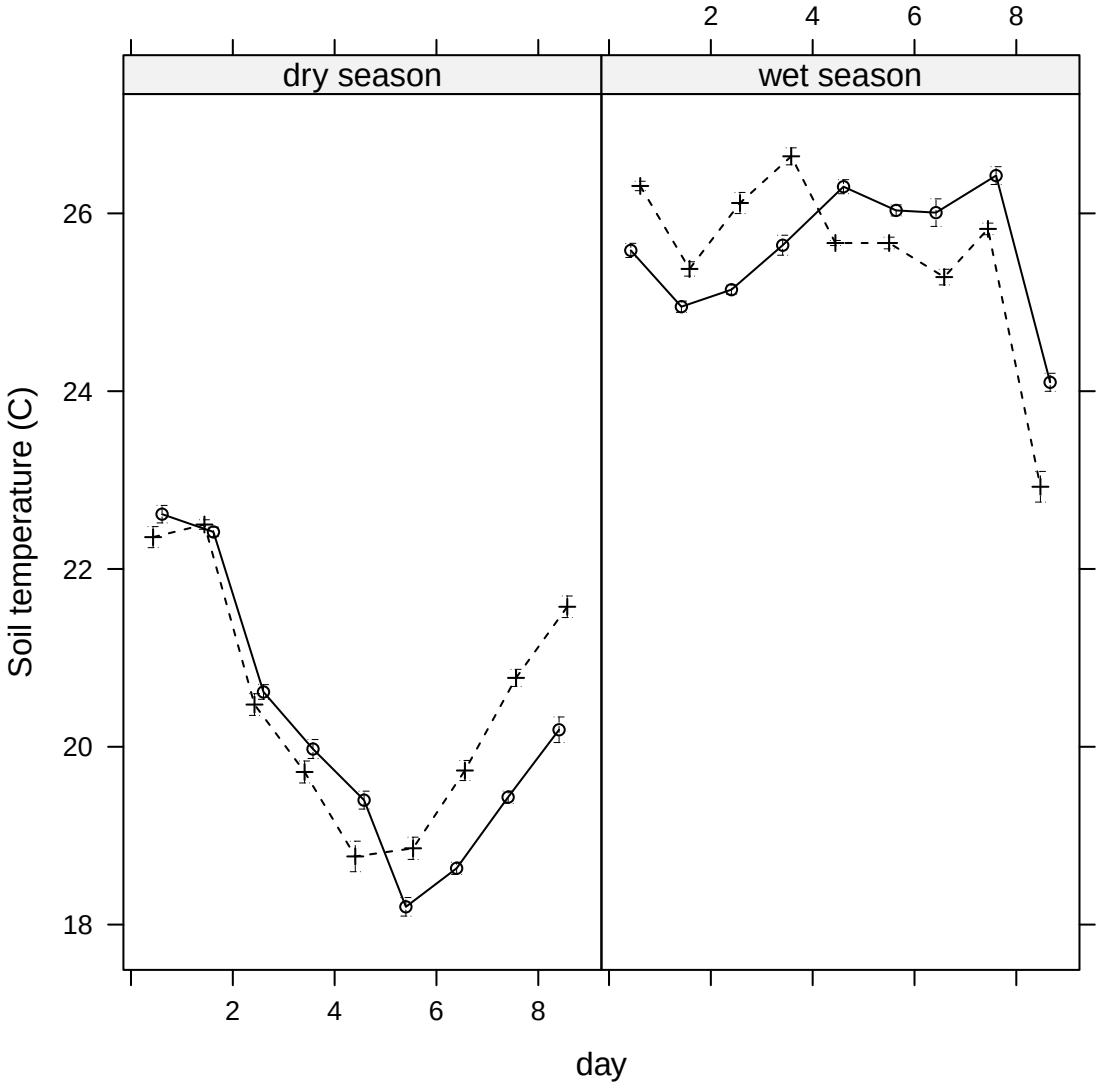

Figure B.2: Daily averaged soil temperature for both sites during dry and wet season. Points (inceptisol, site 1 = ∘, ultisol, site 2 = +) indicate site means and errors bars standard errors.

Appendix C

Supplementary data for Chapter 3

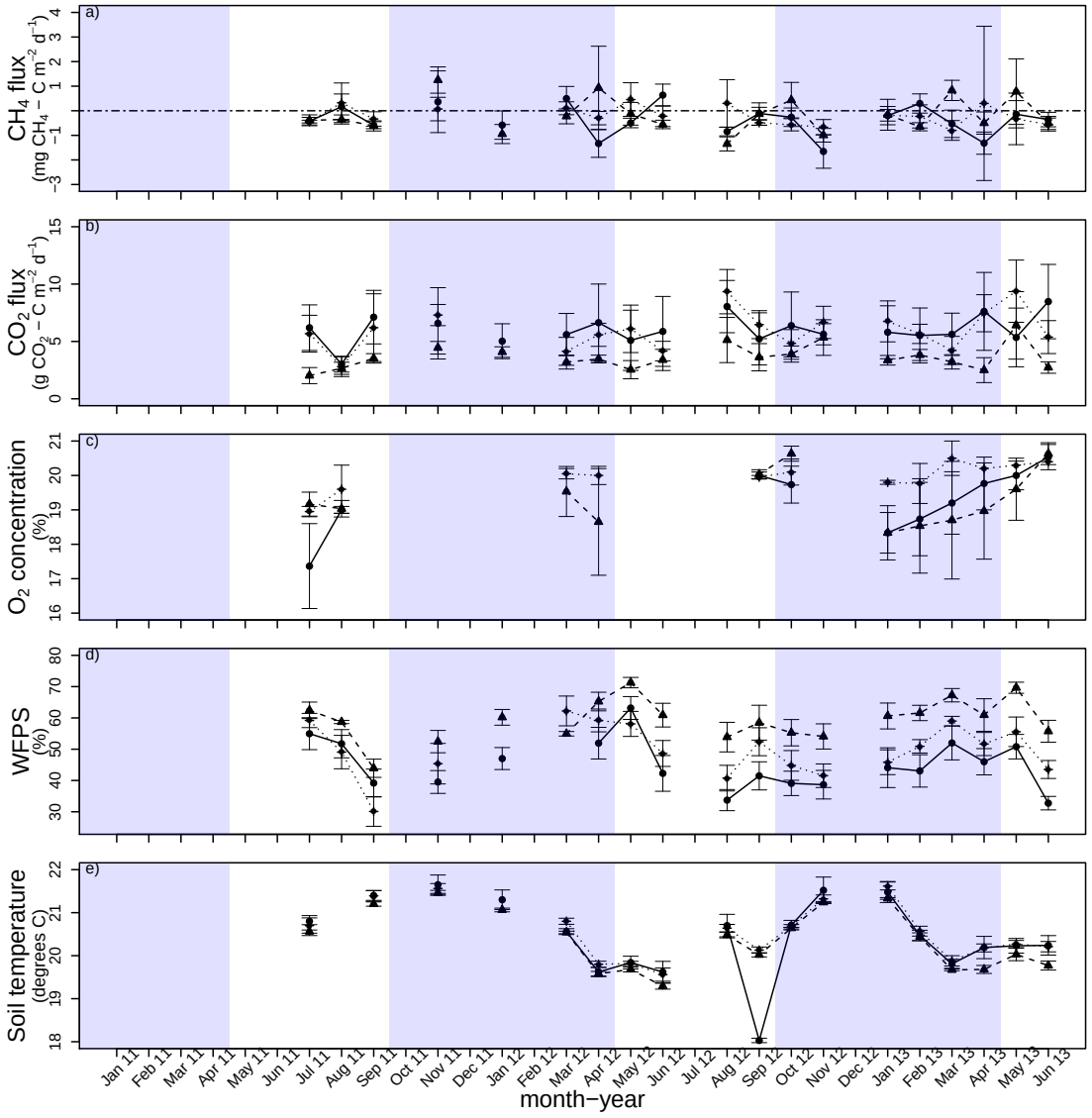

Figure C.1: Premontane forest (Hacienda Villa Carmen): Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) WFPS and c) soil temperature. Shading indicates wet season of October - April.

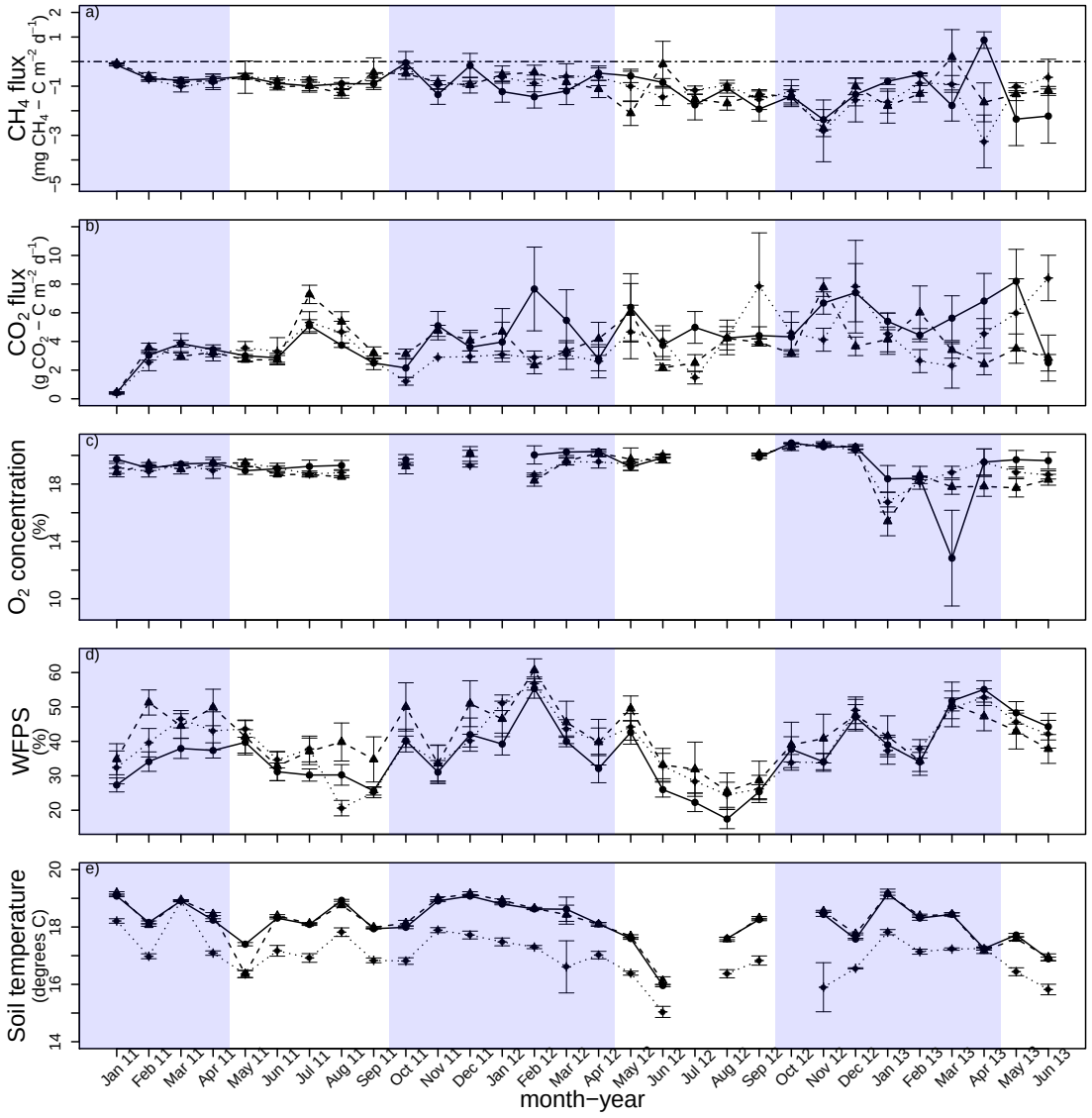

Figure C.2: Lower montane forest (San Pedro): Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) WFPS and c) soil temperature. Shading indicates wet season of October - April.

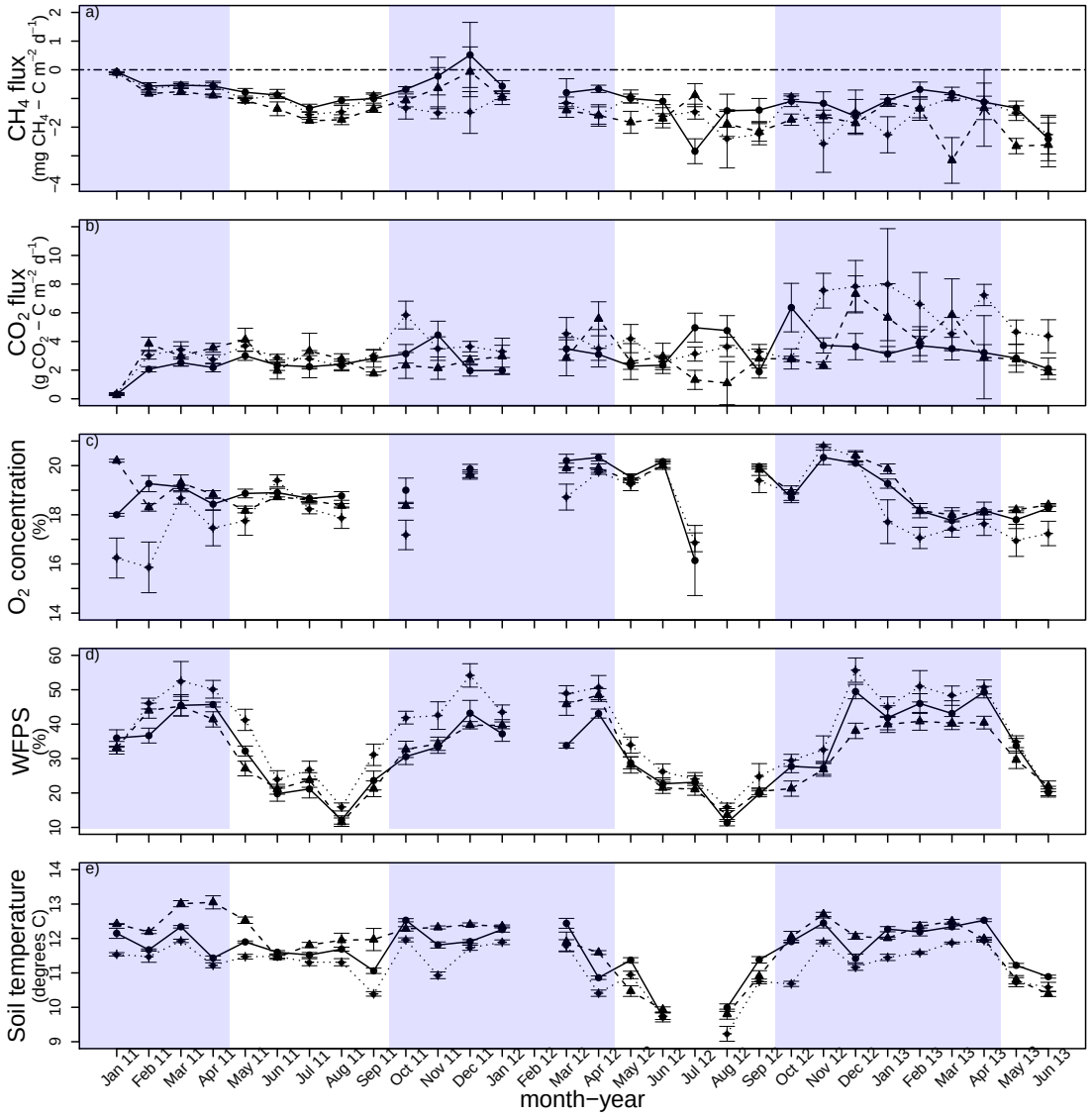

Figure C.3: Upper montane forest (Wayqecha): Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) WFPS and c) soil temperature. Shading indicates wet season of October - April.

Appendix D

Supplementary data for Chapter 4

For the long-term measurements, within plot relationships between of monthly means of CO_2 flux, VWC, soil O_2 concentration, soil temperature and mean net CH_4 flux were investigated through linear regression. Within plots significant relationships between monthly means of net CH_4 flux and CO_2 flux, VWC, soil O_2 concentration and soil temperature were varied. On the ridge, variations in monthly plot mean of net CH_4 flux was best explained by a positively correlation with CO_2 flux ($r^2 = 0.32$). On the slope, variations in monthly plot mean of net CH_4 flux was best explained by a negative correlation with soil O_2 concentration ($r^2 = 0.31$). In the depression, variations in monthly plot mean of net CH_4 flux was best explained by the negative interaction between CO_2 flux and soil O_2 concentration ($r^2 = 0.37$). In the hollow, soil O_2 concentration was excluded from the analysis due to a paucity of observations and variations in montly plot mean of net CH_4 flux was best explained by a negative relationship with CO_2 flux and a positive relationship with soil temperature ($r^2 = 0.46$).

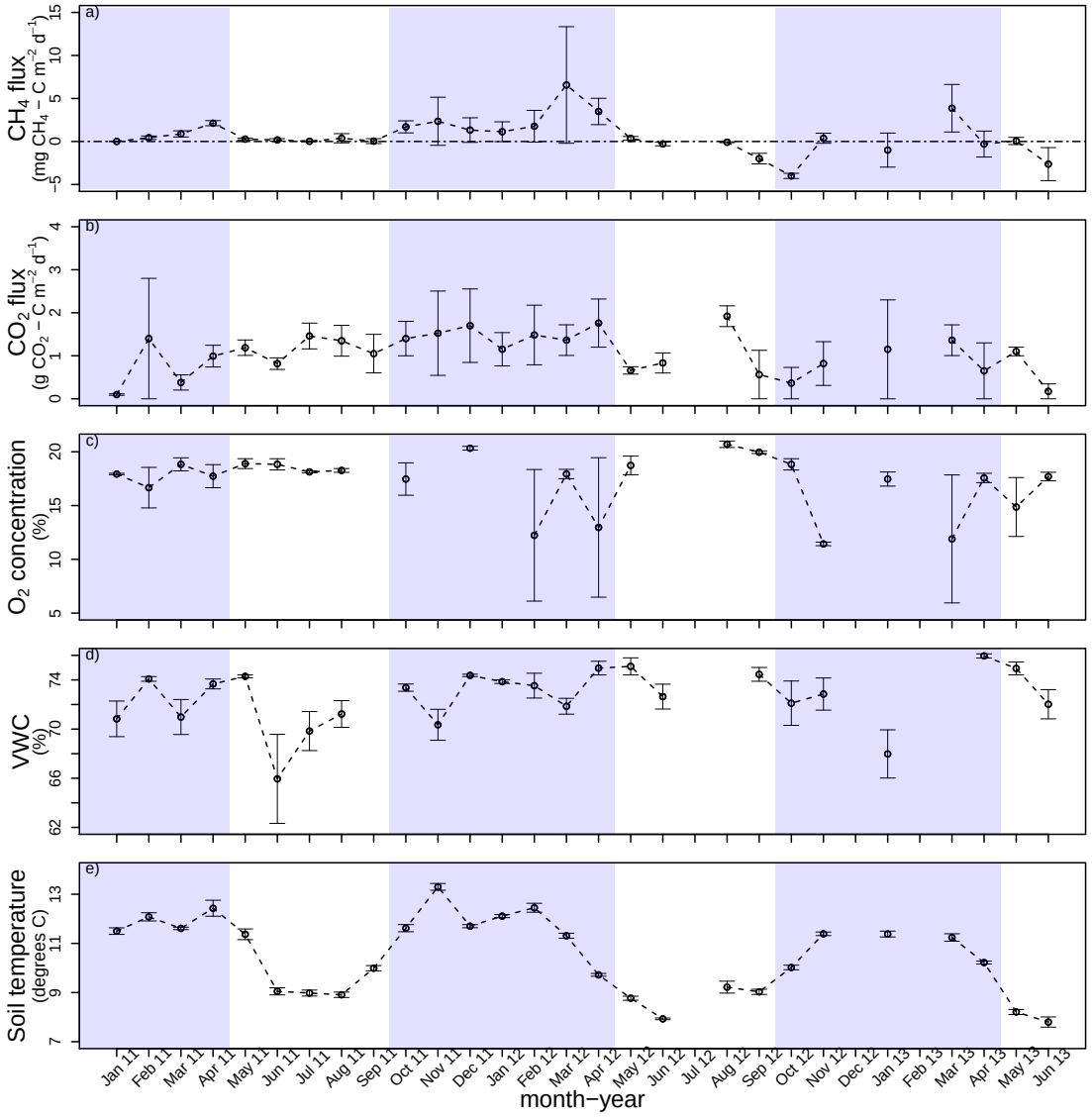

Figure D.1: Ridge: Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) VWC and c) soil temperature. Shading indicates wet season of October - April.

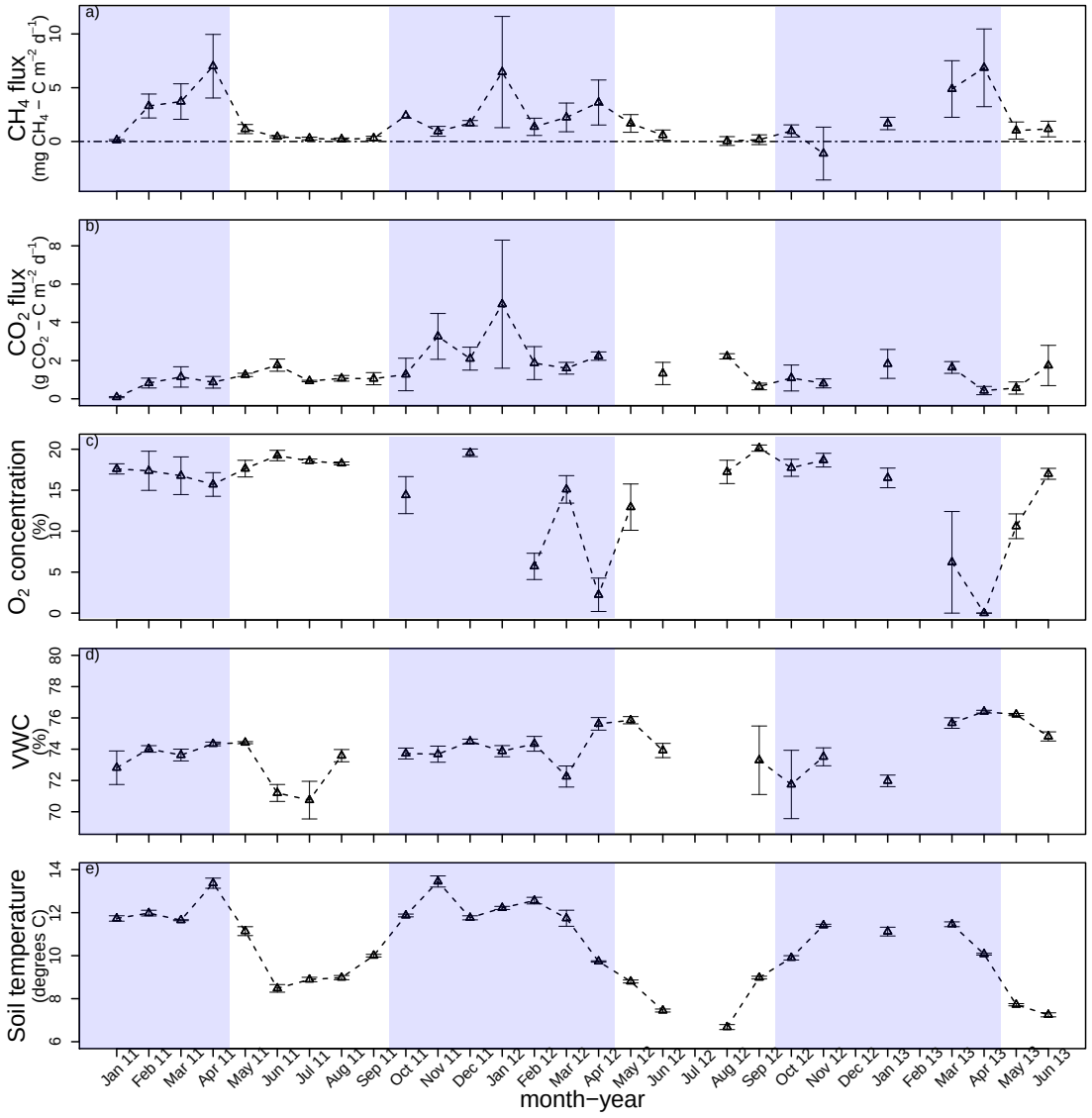

Figure D.2: Slope: Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) VWC and c) soil temperature. Shading indicates wet season of October - April.

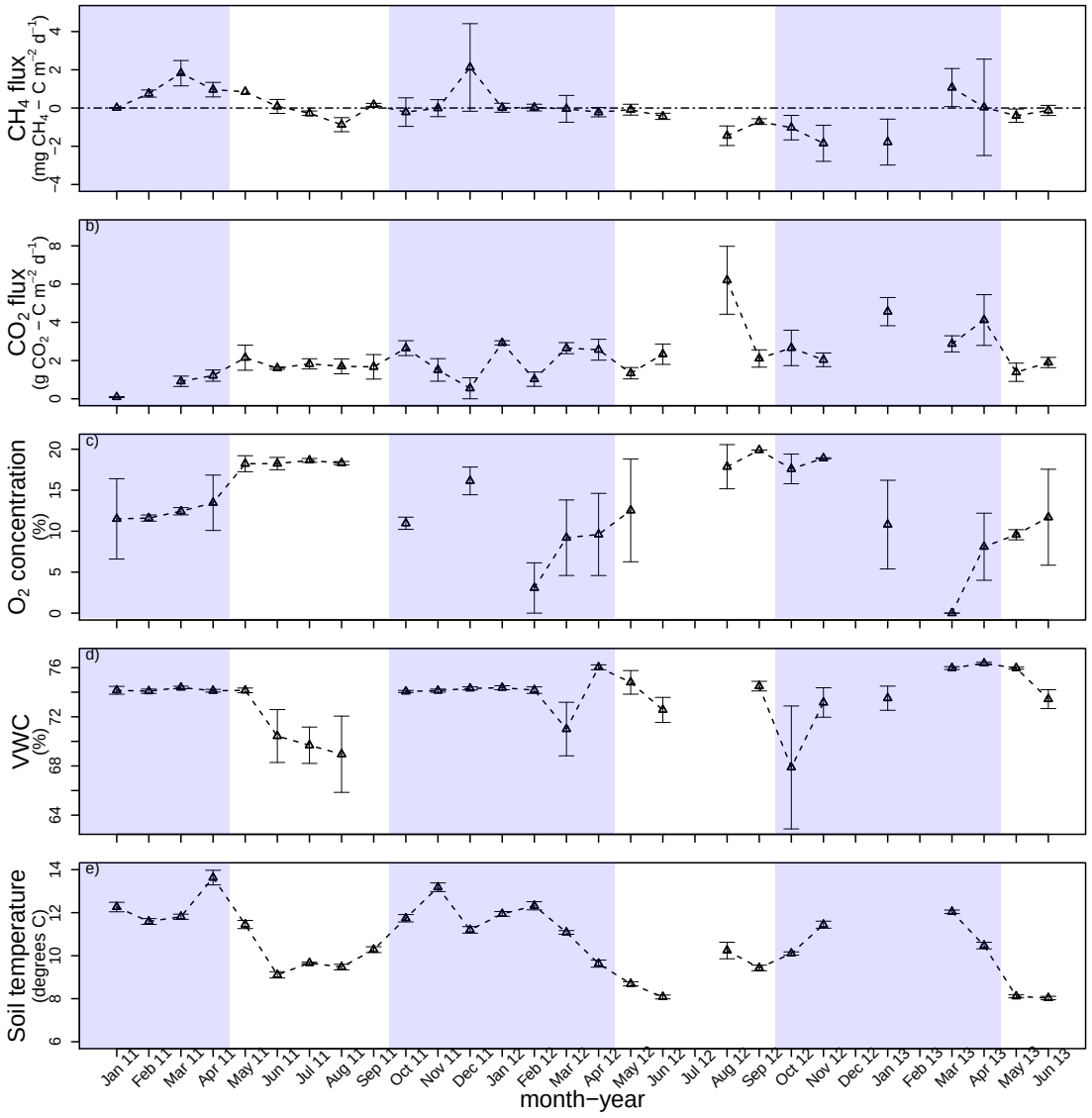

Figure D.3: Depression: Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) VWC and c) soil temperature. Shading indicates wet season of October - April.

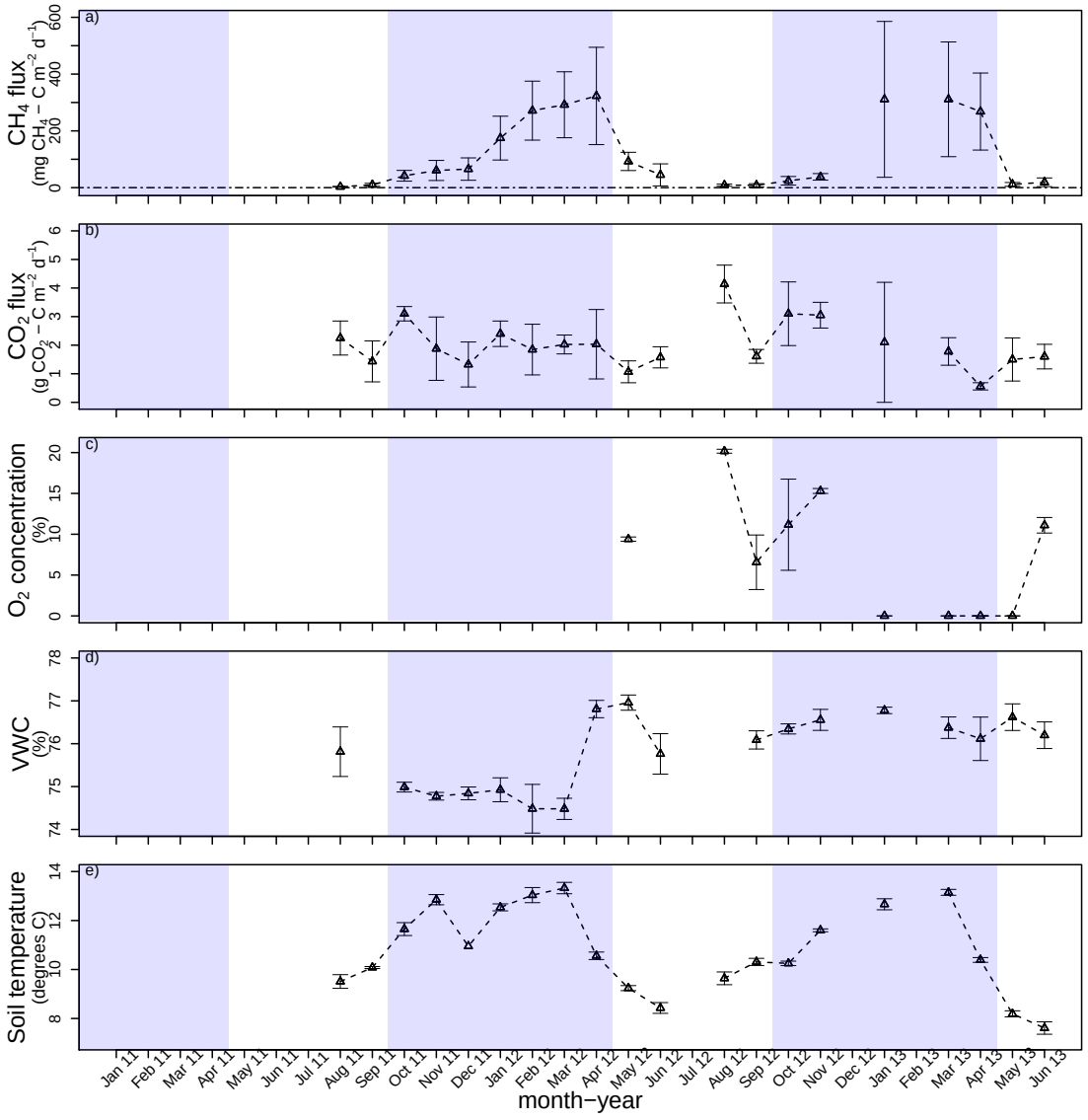

Figure D.4: Hollow: Monthly plot means and standard error bars of a) net CH_4 flux, b) net CO_2 flux, c) soil O_2 concentration, d) VWC and c) soil temperature. Shading indicates wet season of October - April.

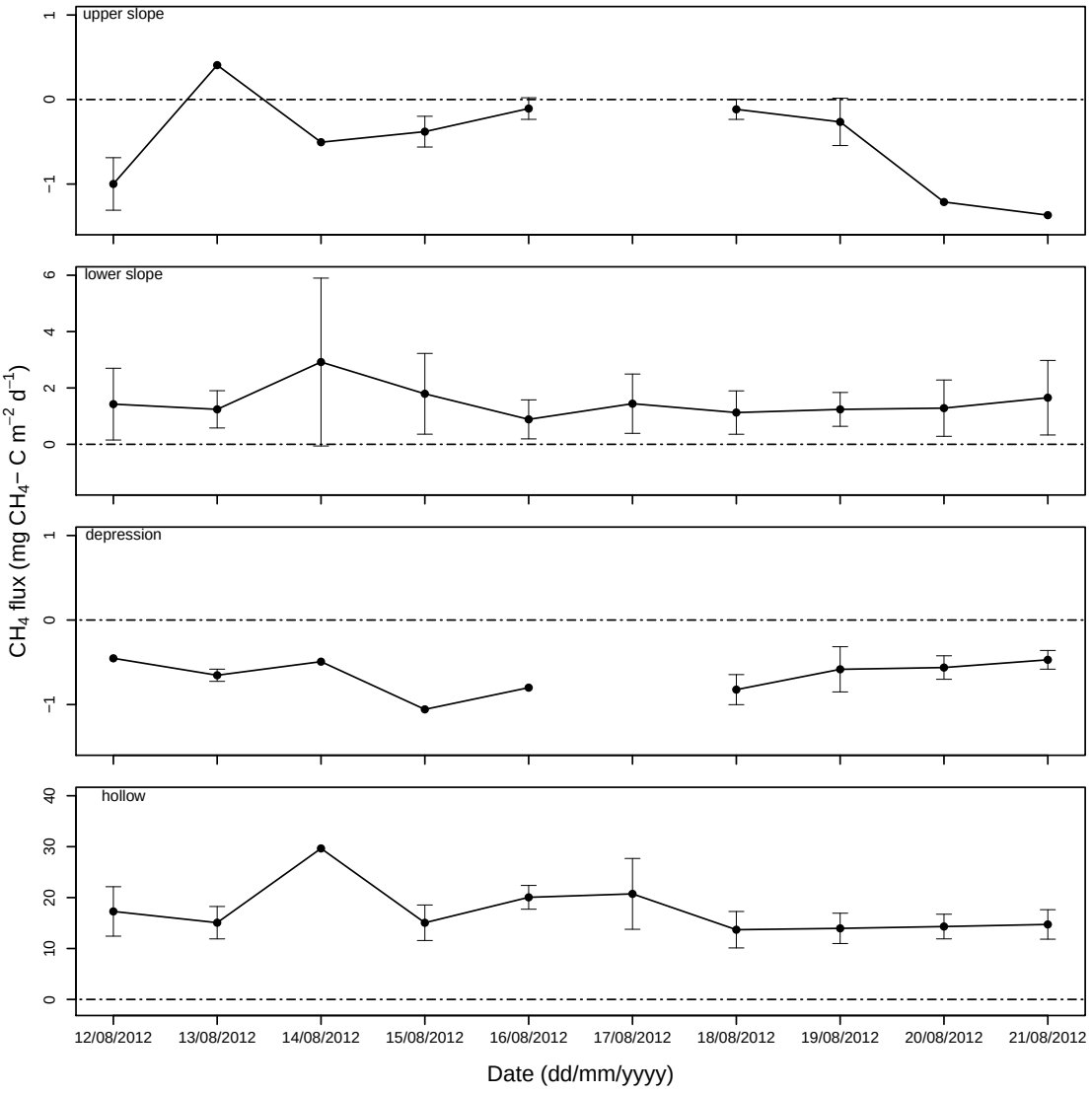

Figure D.5: Dry season campaign, mean and standard errors for net CH_4 fluxes by microform group.

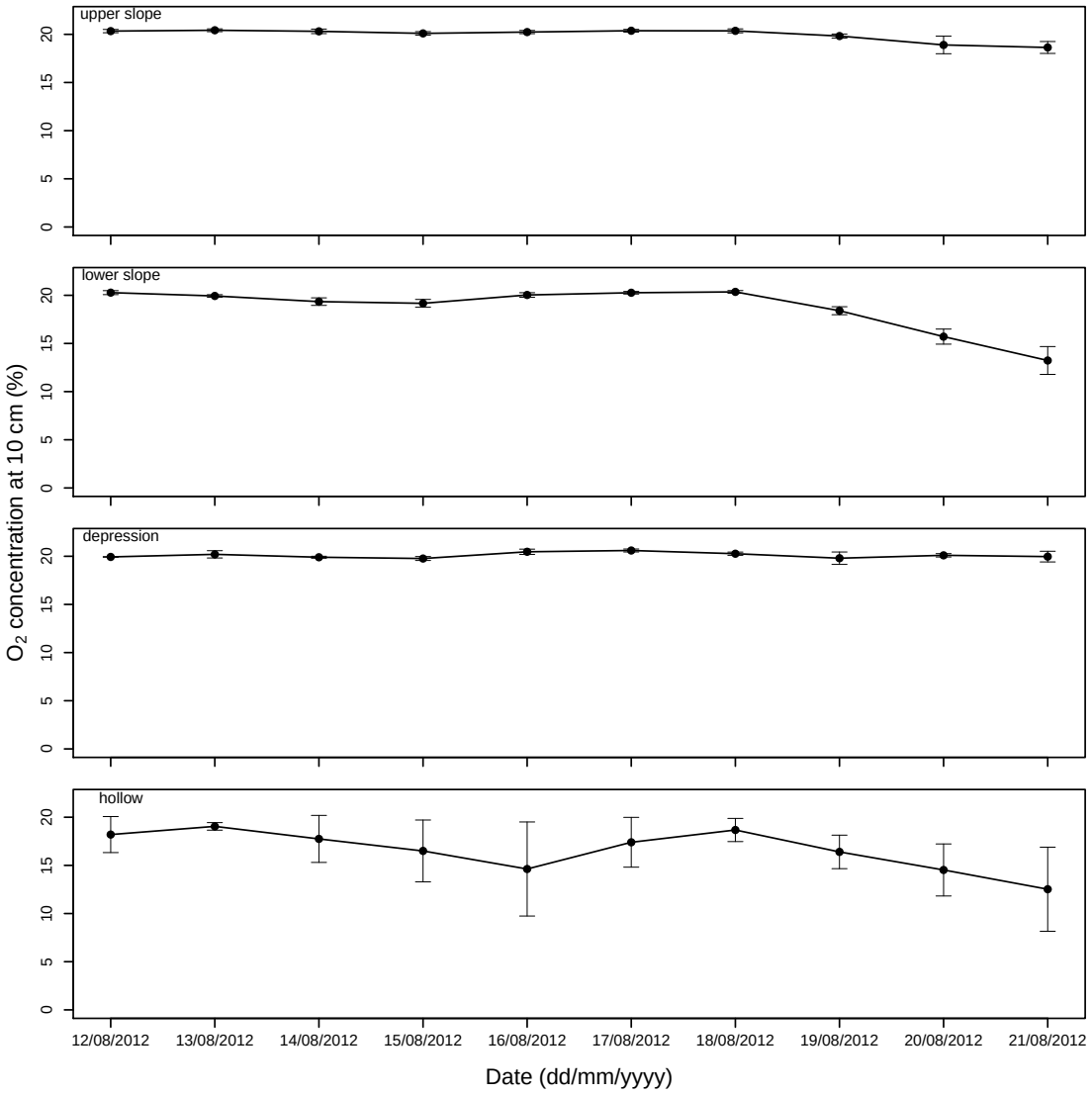

Figure D.6: Dry season campaign, mean and standard errors for soil O_2 concentration by microform group.

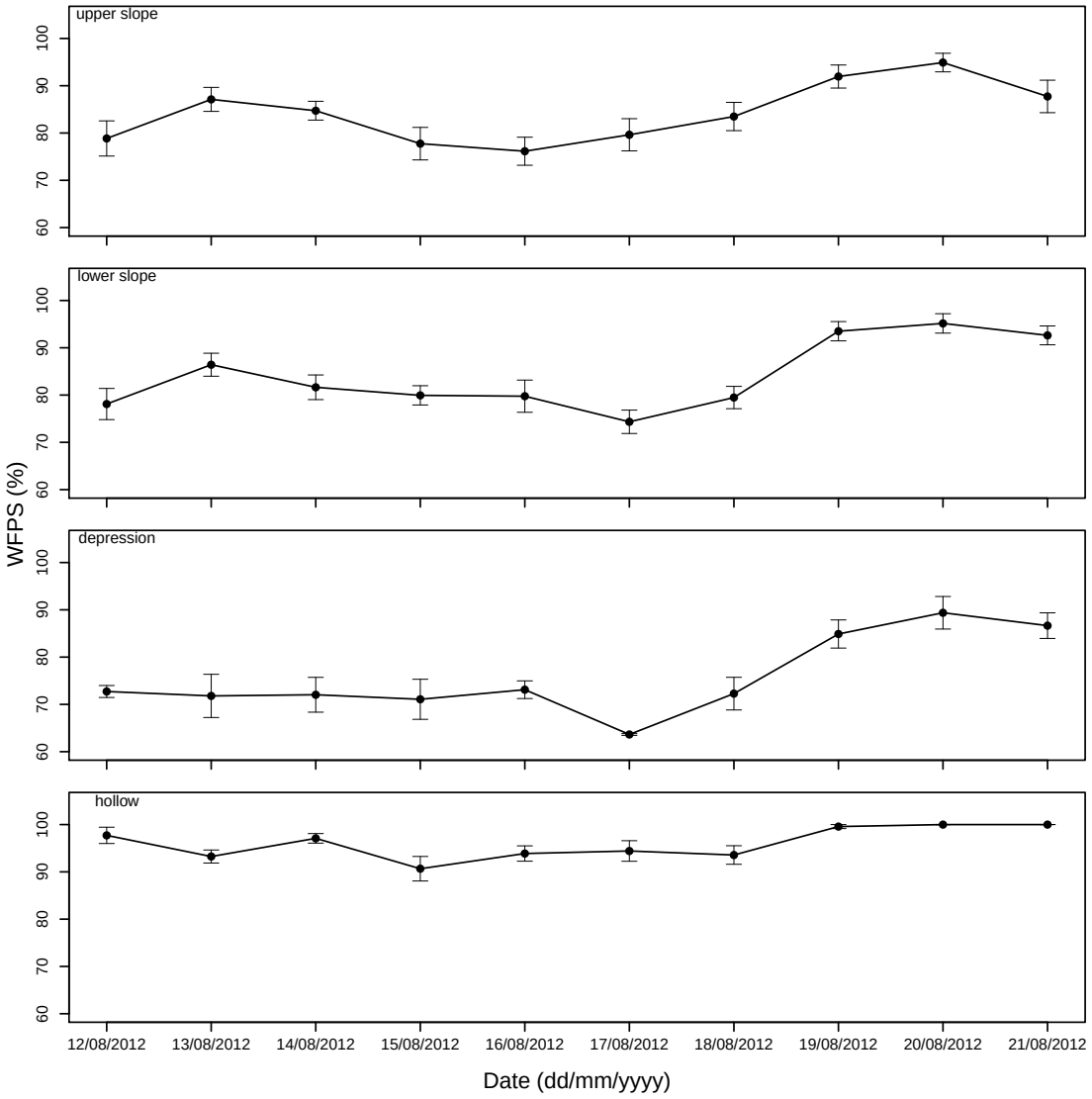

Figure D.7: Dry season campaign, mean and standard errors for WFPS by microform group.

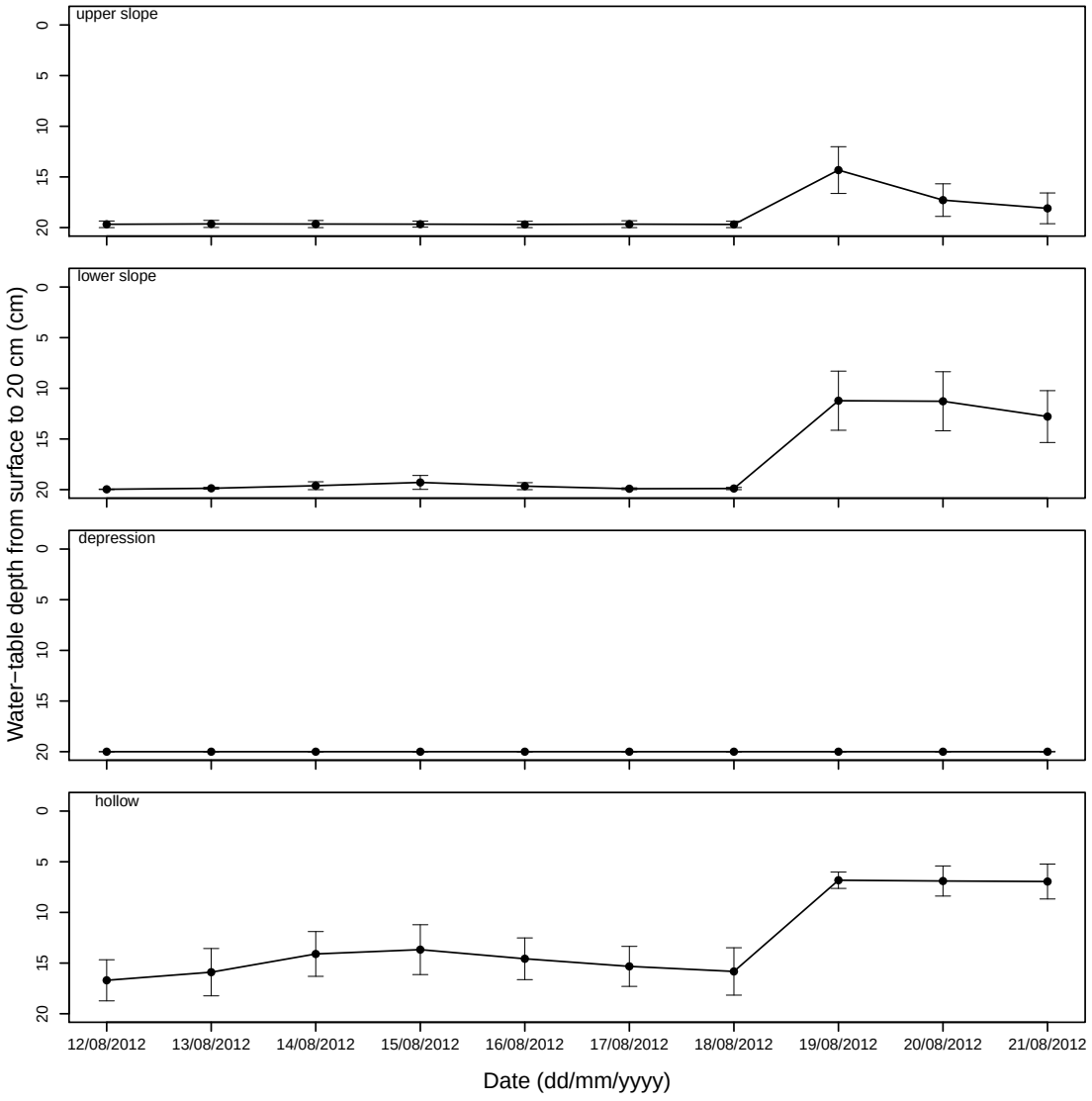

Figure D.8: Dry season campaign, mean and standard errors for water table depth by microform group.

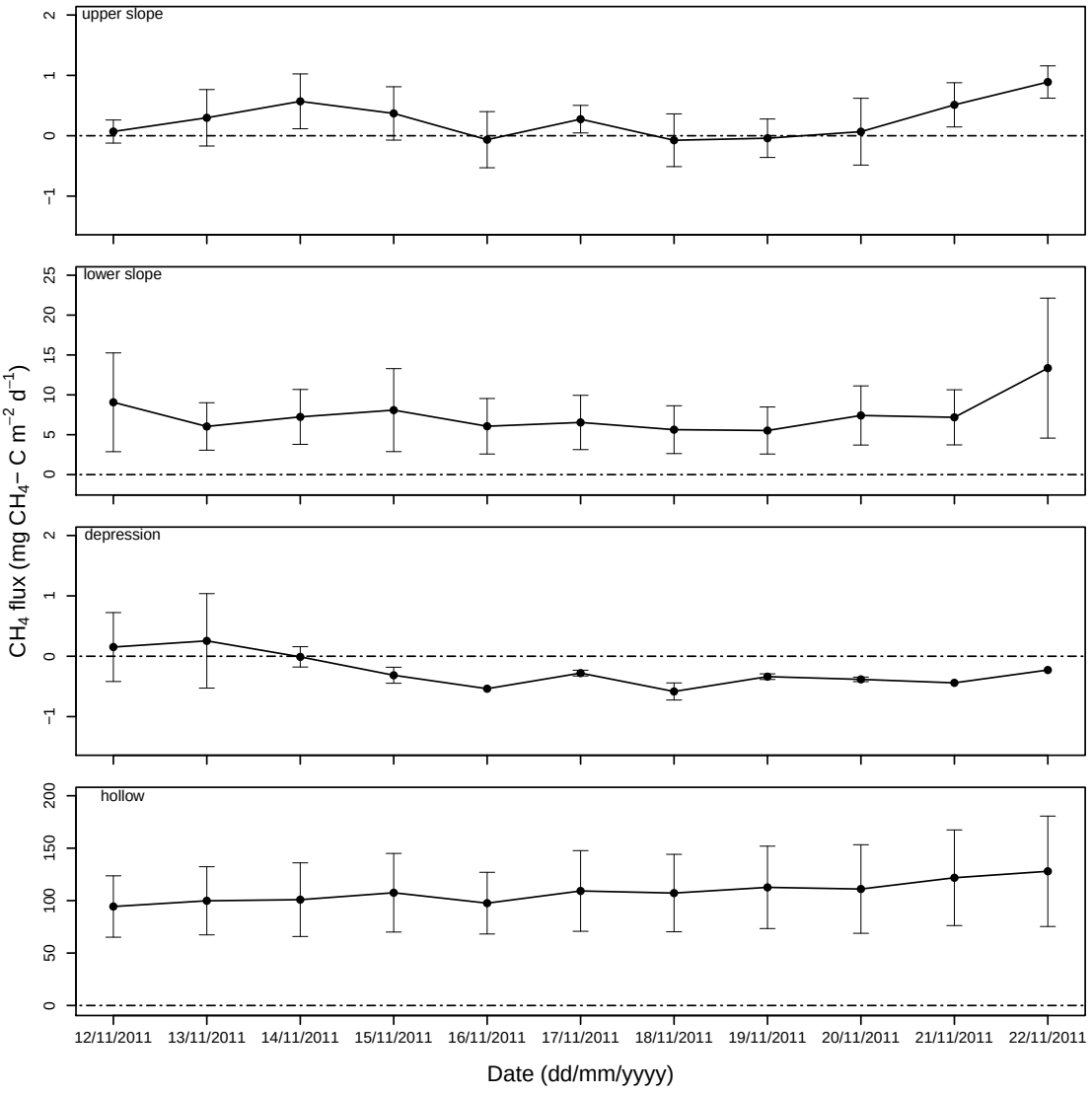

Figure D.9: Wet season campaign, mean and standard errors for net CH_4 fluxes by microform group.

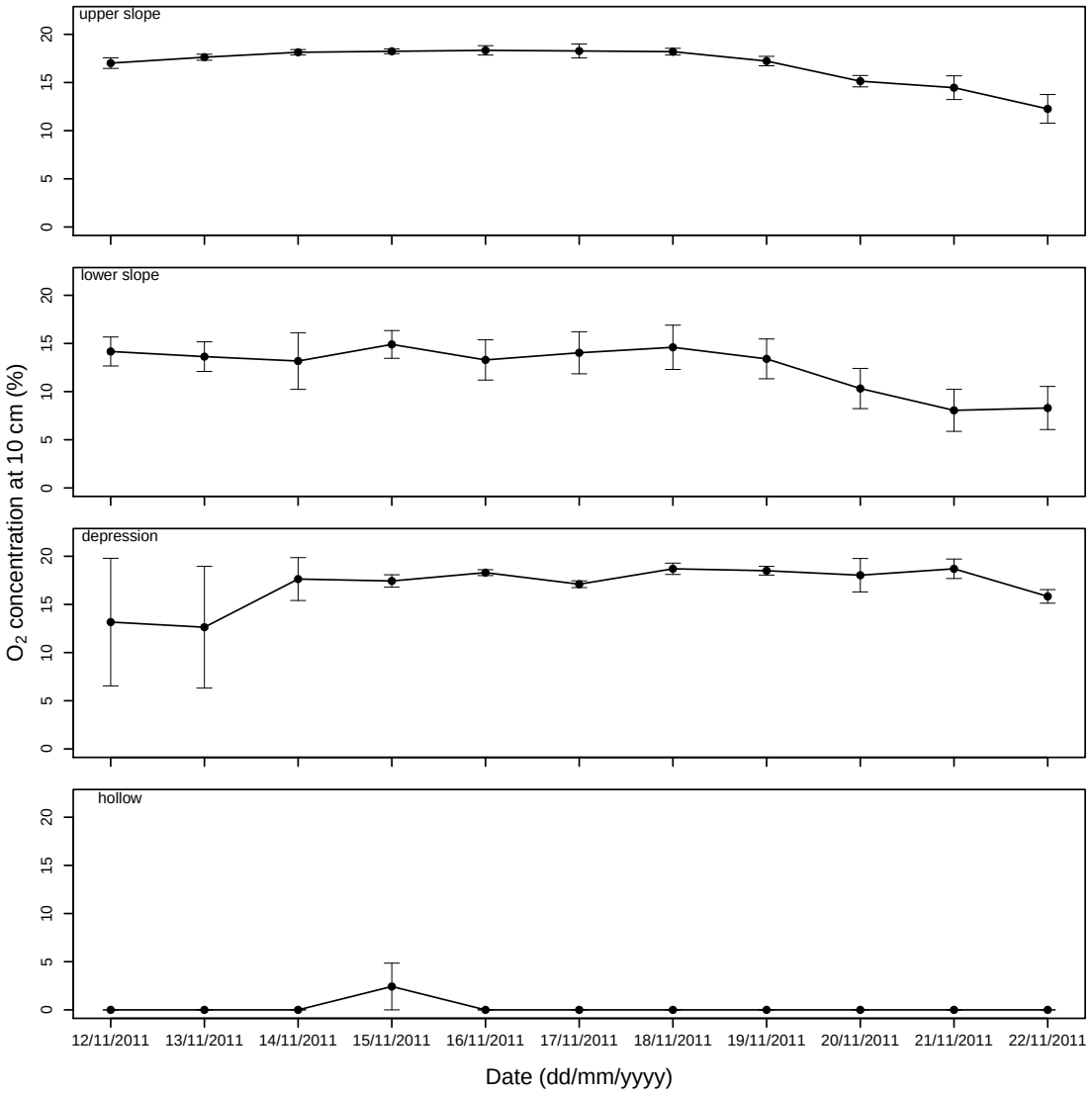

Figure D.10: Wet season campaign, mean and standard errors for soil O_2 concentration by microform group.

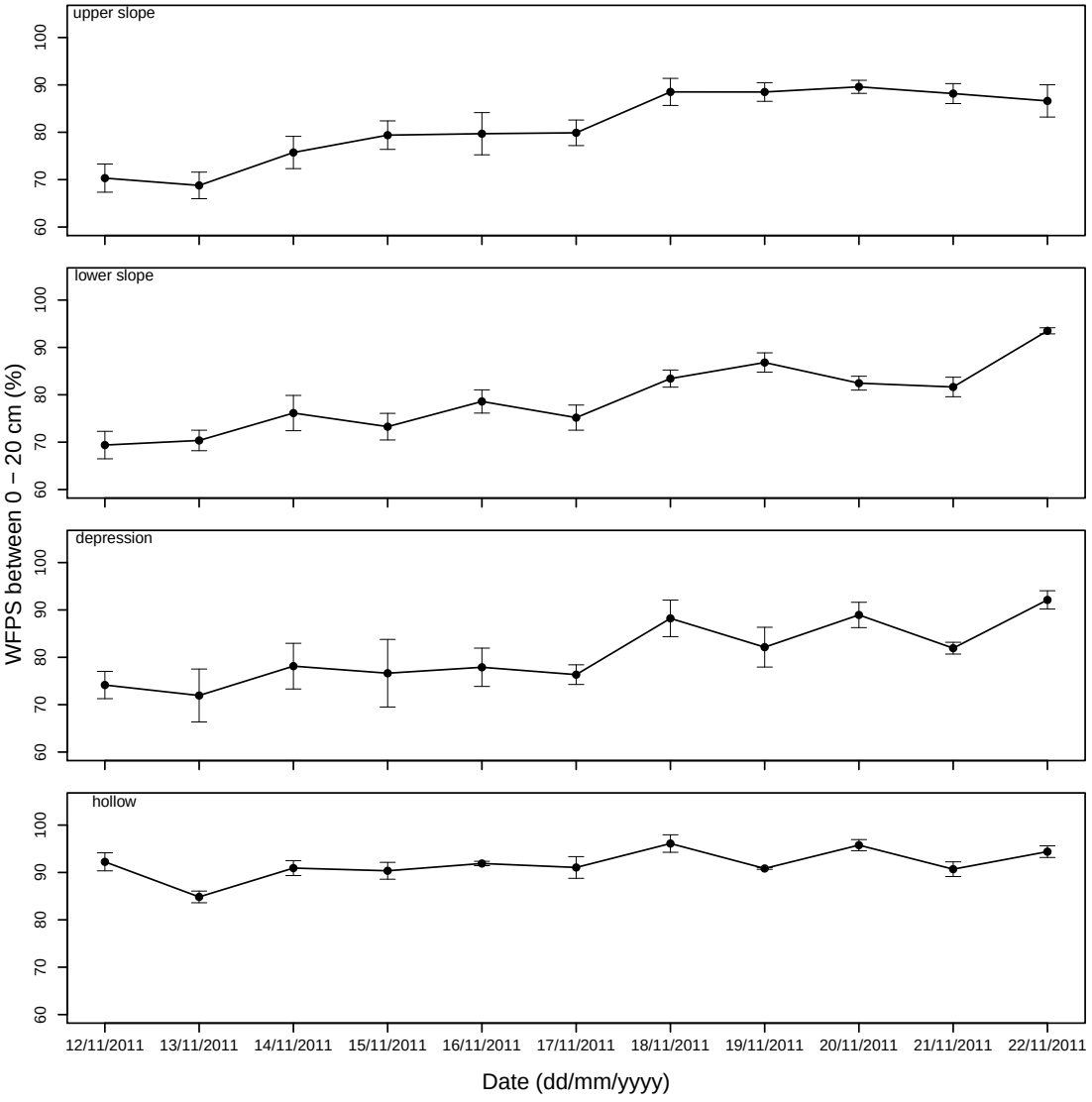

Figure D.11: Wet season campaign, mean and standard errors for WFPS by microform group.

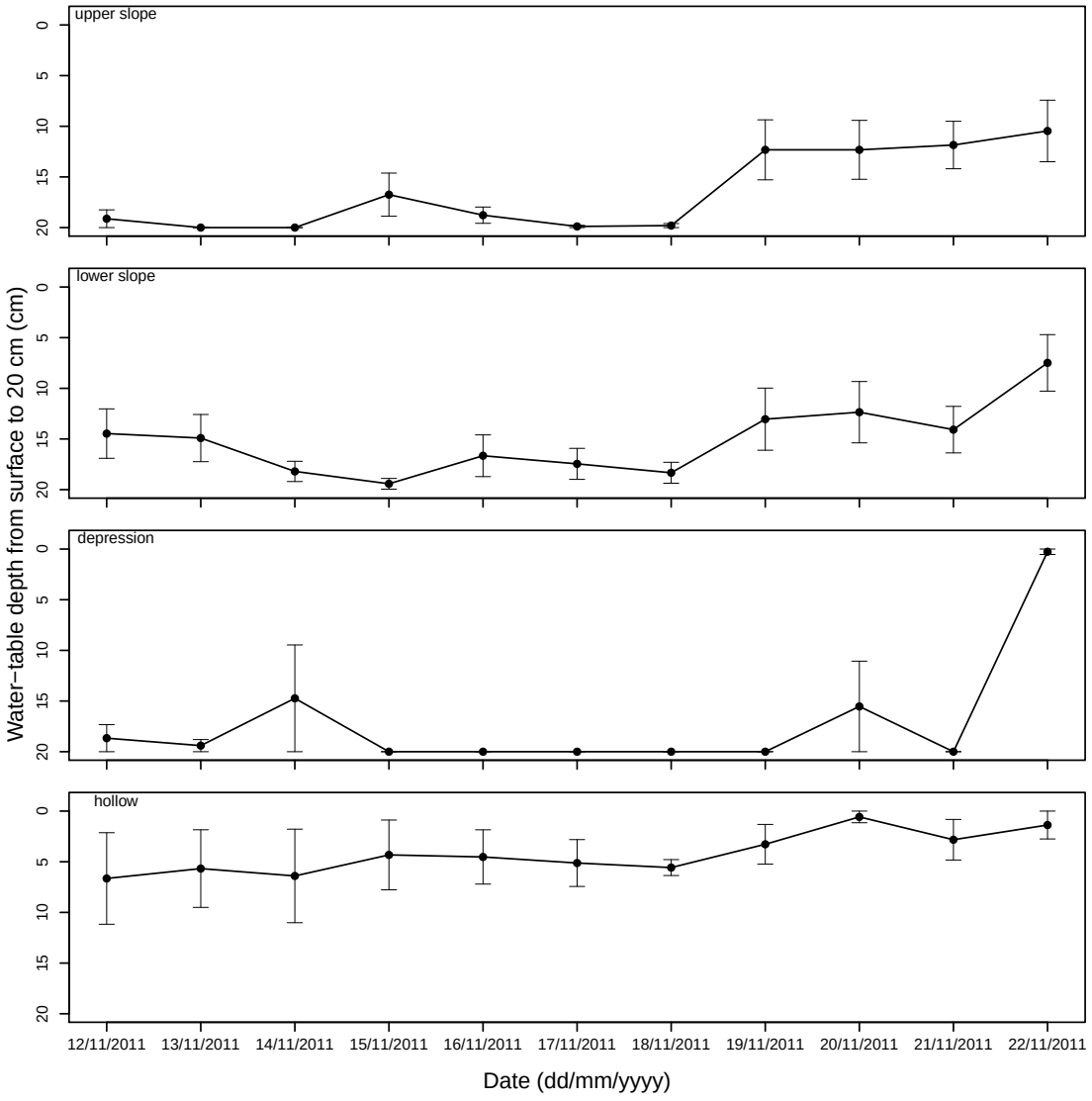

Figure D.12: Wet season campaign, mean and standard errors for water table depth by microform group.

Appendix E

Teh et al., [2014], Methane and nitrous oxide fluxes across an elevation gradient in the tropical Peruvian Andes

A copy of this publication (doi:10.5194/bg-11-2325-2014) is included on the attached CD in PDF format and is available online (http://www.biogeosciences.net/11/2325/2014/bg-11-2325-2014.html) or in print (Biogeosciences, 11, 2325-2339, 2014) from Biogeosciences.